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(54) Title: COMPOSITIONS AND METHODS FOR THERAPY AND DIAGNOSIS OF COLON CANCER

(57) Abstract: Compositions and methods for the therapy and diagnosis of cancer, such as colon cancer, are disclosed. Compositions may comprise one or more colon tumor proteins, immunogenic portions thereof, or polynucleotides that encode such portions. Alternatively, a therapeutic composition may comprise an antigen presenting cell that expresses a colon tumor protein, or a T cell that is specific for cells expressing such a protein. Such compositions may be used, for example, for the prevention and treatment of diseases such as colon cancer. Diagnostic methods based on detecting a colon tumor protein, or mRNA encoding such a protein, in a sample are also provided.

COMPOSITIONS AND METHODS FOR THERAPY AND DIAGNOSIS OF COLON CANCER

TECHNICAL FIELD

The present invention relates generally to therapy and diagnosis of cancer, such as colon cancer. The invention is more specifically related to polypeptides comprising at least a portion of a colon tumor protein, and to polynucleotides encoding such polypeptides. Such polypeptides and polynucleotides may be used in vaccines and pharmaceutical compositions for prevention and treatment of colon cancers, and for the diagnosis and monitoring of such cancers.

10 BACKGROUND OF THE INVENTION

Cancer is a significant health problem throughout the world. Although advances have been made in detection and therapy of cancer, no vaccine or other universally successful method for prevention or treatment is currently available. Current therapies, which are generally based on a combination of chemotherapy or surgery and radiation, continue to prove inadequate in many patients.

Colon cancer is the second most frequently diagnosed malignancy in the United States as well as the second most common cause of cancer death. An estimated 95,600 new cases of colon cancer will be diagnosed in 1998, with an estimated 47,700 deaths. The five-year survival rate for patients with colorectal cancer detected in an early localized stage is 92%; unfortunately, only 37% of colorectal cancer is diagnosed at this stage. The survival rate drops to 64% if the cancer is allowed to spread to adjacent organs or lymph nodes, and to 7% in patients with distant metastases.

The prognosis of colon cancer is directly related to the degree of penetration of the tumor through the bowel wall and the presence or absence of nodal involvement, consequently, early detection and treatment are especially important. Currently, diagnosis is aided by the use of screening assays for fecal occult blood, sigmoidoscopy, colonoscopy and double contrast barium enemas. Treatment regimens are determined by the type and stage of the cancer, and include surgery, radiation therapy and/or chemotherapy. Recurrence following surgery (the most common form

of therapy) is a major problem and is often the ultimate cause of death. In spite of considerable research into therapies for the disease, colon cancer remains difficult to diagnose and treat. In spite of considerable research into therapies for these and other cancers, colon cancer remains difficult to diagnose and treat effectively. Accordingly, there is a need in the art for improved methods for detecting and treating such cancers. The present invention fulfills these needs and further provides other related advantages.

SUMMARY OF THE INVENTION

Briefly stated, the present invention provides compositions and methods for the diagnosis and therapy of cancer, such as colon cancer. In one aspect, the present invention provides polypeptides comprising at least a portion of a colon tumor protein, or a variant thereof. Certain portions and other variants are immunogenic, such that the ability of the variant to react with antigen-specific antisera is not substantially diminished. Within certain embodiments, the polypeptide comprises a sequence that is encoded by a polynucleotide sequence selected from the group consisting of: (a) sequences recited in SEQ ID NOs:1-1556; (b) variants of a sequence recited in SEQ ID NO: 1-1556; and (c) complements of a sequence of (a) or (b).

The present invention further provides polynucleotides that encode a polypeptide as described above, or a portion thereof (such as a portion encoding at least 15 amino acid residues of a colon tumor protein), expression vectors comprising such polynucleotides and host cells transformed or transfected with such expression vectors.

Within other aspects, the present invention provides pharmaceutical compositions comprising a polypeptide or polynucleotide as described above and a physiologically acceptable carrier.

Within a related aspect of the present invention, vaccines for prophylactic or therapeutic use are provided. Such vaccines comprise a polypeptide or polynucleotide as described above and an immunostimulant.

The present invention further provides pharmaceutical compositions that comprise: (a) an antibody or antigen-binding fragment thereof that specifically binds to a colon tumor protein; and (b) a physiologically acceptable carrier.

Within further aspects, the present invention provides pharmaceutical compositions comprising: (a) an antigen presenting cell that expresses a polypeptide as described above and (b) a pharmaceutically acceptable carrier or excipient. Antigen presenting cells include dendritic cells, macrophages, monocytes, fibroblasts and B cells.

Within related aspects, vaccines are provided that comprise: (a) an antigen presenting cell that expresses a polypeptide as described above and (b) an immunostimulant.

The present invention further provides, in other aspects, fusion proteins that comprise at least one polypeptide as described above, as well as polynucleotides encoding such fusion proteins.

Within related aspects, pharmaceutical compositions comprising a fusion protein, or a polynucleotide encoding a fusion protein, in combination with a physiologically acceptable carrier are provided.

Vaccines are further provided, within other aspects, that comprise a fusion protein, or a polynucleotide encoding a fusion protein, in combination with an immunostimulant.

Within further aspects, the present invention provides methods for inhibiting the development of a cancer in a patient, comprising administering to a patient a pharmaceutical composition or vaccine as recited above. The patient may be afflicted with colon cancer, in which case the methods provide treatment for the disease, or patient considered at risk for such a disease may be treated prophylactically.

The present invention further provides, within other aspects, methods for removing tumor cells from a biological sample, comprising contacting a biological sample with T cells that specifically react with a colon tumor protein, wherein the step of contacting is performed under conditions and for a time sufficient to permit the removal of cells expressing the protein from the sample.

Within related aspects, methods are provided for inhibiting the development of a cancer in a patient, comprising administering to a patient a biological sample treated as described above.

Methods are further provided, within other aspects, for stimulating and/or expanding T cells specific for a colon tumor protein, comprising contacting T cells with one or more of: (i) a polypeptide as described above; (ii) a polynucleotide encoding such a polypeptide; and/or (iii) an antigen presenting cell that expresses such a polypeptide; under conditions and for a time sufficient to permit the stimulation and/or expansion of T cells. Isolated T cell populations comprising T cells prepared as described above are also provided.

Within further aspects, the present invention provides methods for inhibiting the development of a cancer in a patient, comprising administering to a patient an effective amount of a T cell population as described above.

The present invention further provides methods for inhibiting the development of a cancer in a patient, comprising the steps of: (a) incubating CD4⁺ and/or CD8⁺ T cells isolated from a patient with one or more of: (i) a polypeptide comprising at least an immunogenic portion of a colon tumor protein; (ii) a polynucleotide encoding such a polypeptide; and (iii) an antigen-presenting cell that expressed such a polypeptide; and (b) administering to the patient an effective amount of the proliferated T cells, and thereby inhibiting the development of a cancer in the patient. Proliferated cells may, but need not, be cloned prior to administration to the patient.

Within further aspects, the present invention provides methods for determining the presence or absence of a cancer in a patient, comprising: (a) contacting a biological sample obtained from a patient with a binding agent that binds to a polypeptide as recited above; (b) detecting in the sample an amount of polypeptide that binds to the binding agent; and (c) comparing the amount of polypeptide with a predetermined cut-off value, and therefrom determining the presence or absence of a cancer in the patient. Within preferred embodiments, the binding agent is an antibody, more preferably a monoclonal antibody. The cancer may be colon cancer.

The present invention also provides, within other aspects, methods for monitoring the progression of a cancer in a patient. Such methods comprise the steps of: (a) contacting a biological sample obtained from a patient at a first point in time with a binding agent that binds to a polypeptide as recited above; (b) detecting in the

sample an amount of polypeptide that binds to the binding agent; (c) repeating steps (a) and (b) using a biological sample obtained from the patient at a subsequent point in time; and (d) comparing the amount of polypeptide detected in step (c) with the amount detected in step (b) and therefrom monitoring the progression of the cancer in the patient.

The present invention further provides, within other aspects, methods for determining the presence or absence of a cancer in a patient, comprising the steps of: (a) contacting a biological sample obtained from a patient with an oligonucleotide that hybridizes to a polynucleotide that encodes a colon tumor protein; (b) detecting in the sample a level of a polynucleotide, preferably mRNA, that hybridizes to the oligonucleotide; and (c) comparing the level of polynucleotide that hybridizes to the oligonucleotide with a predetermined cut-off value, and therefrom determining the presence or absence of a cancer in the patient. Within certain embodiments, the amount of mRNA is detected via polymerase chain reaction using, for example, at least one oligonucleotide primer that hybridizes to a polynucleotide encoding a polypeptide as recited above, or a complement of such a polynucleotide. Within other embodiments, the amount of mRNA is detected using a hybridization technique, employing an oligonucleotide probe that hybridizes to a polynucleotide that encodes a polypeptide as recited above, or a complement of such a polynucleotide.

In related aspects, methods are provided for monitoring the progression of a cancer in a patient, comprising the steps of: (a) contacting a biological sample obtained from a patient with an oligonucleotide that hybridizes to a polynucleotide that encodes a colon tumor protein; (b) detecting in the sample an amount of a polynucleotide that hybridizes to the oligonucleotide; (c) repeating steps (a) and (b) using a biological sample obtained from the patient at a subsequent point in time; and (d) comparing the amount of polynucleotide detected in step (c) with the amount detected in step (b) and therefrom monitoring the progression of the cancer in the patient.

Within further aspects, the present invention provides antibodies, such as monoclonal antibodies, that bind to a polypeptide as described above, as well as

diagnostic kits comprising such antibodies. Diagnostic kits comprising one or more oligonucleotide probes or primers as described above are also provided.

These and other aspects of the present invention will become apparent upon reference to the following detailed description. All references disclosed herein are
5 hereby incorporated by reference in their entirety as if each was incorporated individually.

DETAILED DESCRIPTION OF THE INVENTION

As noted above, the present invention is generally directed to compositions and methods for the therapy and diagnosis of cancer, such as colon
10 cancer. The compositions described herein may include colon tumor polypeptides, polynucleotides encoding such polypeptides, binding agents such as antibodies, antigen presenting cells (APCs) and/or immune system cells (*e.g.*, T cells). Polypeptides of the present invention generally comprise at least a portion (such as an immunogenic portion) of a colon tumor protein or a variant thereof. A "colon tumor protein" is a
15 protein that is expressed in colon tumor cells at a level that is at least two fold, and preferably at least five fold, greater than the level of expression in a normal tissue, as determined using a representative assay provided herein. Certain colon tumor proteins are tumor proteins that react detectably (within an immunoassay, such as an ELISA or Western blot) with antisera of a patient afflicted with colon cancer. Polynucleotides of
20 the subject invention generally comprise a DNA or RNA sequence that encodes all or a portion of such a polypeptide, or that is complementary to such a sequence. Antibodies are generally immune system proteins, or antigen-binding fragments thereof, that are capable of binding to a polypeptide as described above. Antigen presenting cells include dendritic cells, macrophages, monocytes, fibroblasts and B-cells that express a
25 polypeptide as described above. T cells that may be employed within such compositions are generally T cells that are specific for a polypeptide as described above.

The present invention is based on the discovery of human colon tumor proteins. Sequences of polynucleotides encoding specific tumor proteins are provided
30 in SEQ ID NOs:1-1556.

COLON TUMOR PROTEIN POLYNUCLEOTIDES

Any polynucleotide that encodes a colon tumor protein or a portion or other variant thereof as described herein is encompassed by the present invention. Preferred polynucleotides comprise at least 15 consecutive nucleotides, preferably at least 30 consecutive nucleotides and more preferably at least 45 consecutive nucleotides, that encode a portion of a colon tumor protein. More preferably, a polynucleotide encodes an immunogenic portion of a colon tumor protein. Polynucleotides complementary to any such sequences are also encompassed by the present invention. Polynucleotides may be single-stranded (coding or antisense) or double-stranded, and may be DNA (genomic, cDNA or synthetic) or RNA molecules. RNA molecules include HnRNA molecules, which contain introns and correspond to a DNA molecule in a one-to-one manner, and mRNA molecules, which do not contain introns. Additional coding or non-coding sequences may, but need not, be present within a polynucleotide of the present invention, and a polynucleotide may, but need not, be linked to other molecules and/or support materials.

Polynucleotides may comprise a native sequence (*i.e.*, an endogenous sequence that encodes a colon tumor protein or a portion thereof) or may comprise a variant of such a sequence. Polynucleotide variants may contain one or more substitutions, additions, deletions and/or insertions such that the immunogenicity of the encoded polypeptide is not diminished, relative to a native tumor protein. The effect on the immunogenicity of the encoded polypeptide may generally be assessed as described herein. Variants preferably exhibit at least about 70% identity, more preferably at least about 80% identity and most preferably at least about 90% identity to a polynucleotide sequence that encodes a native colon tumor protein or a portion thereof. The term "variants" also encompasses homologous genes of xenogenic origin.

Two polynucleotide or polypeptide sequences are said to be "identical" if the sequence of nucleotides or amino acids in the two sequences is the same when aligned for maximum correspondence as described below. Comparisons between two sequences are typically performed by comparing the sequences over a comparison window to identify and compare local regions of sequence similarity. A "comparison window" as used herein, refers to a segment of at least about 20 contiguous positions,

usually 30 to about 75, 40 to about 50, in which a sequence may be compared to a reference sequence of the same number of contiguous positions after the two sequences are optimally aligned.

Optimal alignment of sequences for comparison may be conducted using the Megalign program in the Lasergene suite of bioinformatics software (DNASTAR, Inc., Madison, WI), using default parameters. This program embodies several alignment schemes described in the following references: Dayhoff, M.O. (1978) A model of evolutionary change in proteins – Matrices for detecting distant relationships. In Dayhoff, M.O. (ed.) Atlas of Protein Sequence and Structure, National Biomedical Research Foundation, Washington DC Vol. 5, Suppl. 3, pp. 345-358; Hein J. (1990) Unified Approach to Alignment and Phylogenies pp. 626-645 *Methods in Enzymology* vol. 183, Academic Press, Inc., San Diego, CA; Higgins, D.G. and Sharp, P.M. (1989) *CABIOS* 5:151-153; Myers, E.W. and Muller W. (1988) *CABIOS* 4:11-17; Robinson, E.D. (1971) *Comb. Theor* 11:105; Santou, N. Nes, M. (1987) *Mol. Biol. Evol.* 4:406-425; Sneath, P.H.A. and Sokal, R.R. (1973) *Numerical Taxonomy – the Principles and Practice of Numerical Taxonomy*, Freeman Press, San Francisco, CA; Wilbur, W.J. and Lipman, D.J. (1983) *Proc. Natl. Acad. Sci. USA* 80:726-730.

Preferably, the “percentage of sequence identity” is determined by comparing two optimally aligned sequences over a window of comparison of at least 20 positions, wherein the portion of the polynucleotide or polypeptide sequence in the comparison window may comprise additions or deletions (*i.e.*, gaps) of 20 percent or less, usually 5 to 15 percent, or 10 to 12 percent, as compared to the reference sequences (which does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical nucleic acid bases or amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the reference sequence (*i.e.*, the window size) and multiplying the results by 100 to yield the percentage of sequence identity.

Variants may also, or alternatively, be substantially homologous to a native gene, or a portion or complement thereof. Such polynucleotide variants are capable of hybridizing under moderately stringent conditions to a naturally occurring

DNA sequence encoding a native colon tumor protein (or a complementary sequence). Suitable moderately stringent conditions include prewashing in a solution of 5 X SSC, 0.5% SDS, 1.0 mM EDTA (pH 8.0); hybridizing at 50°C-65°C, 5 X SSC, overnight; followed by washing twice at 65°C for 20 minutes with each of 2X, 0.5X and 0.2X SSC containing 0.1% SDS.

It will be appreciated by those of ordinary skill in the art that, as a result of the degeneracy of the genetic code, there are many nucleotide sequences that encode a polypeptide as described herein. Some of these polynucleotides bear minimal homology to the nucleotide sequence of any native gene. Nonetheless, polynucleotides that vary due to differences in codon usage are specifically contemplated by the present invention. Further, alleles of the genes comprising the polynucleotide sequences provided herein are within the scope of the present invention. Alleles are endogenous genes that are altered as a result of one or more mutations, such as deletions, additions and/or substitutions of nucleotides. The resulting mRNA and protein may, but need not, have an altered structure or function. Alleles may be identified using standard techniques (such as hybridization, amplification and/or database sequence comparison).

Polynucleotides may be prepared using any of a variety of techniques. For example, a polynucleotide may be identified, as described in more detail below, by screening a microarray of cDNAs for tumor-associated expression (*i.e.*, expression that is at least two fold greater in a colon tumor than in normal tissue, as determined using a representative assay provided herein). Such screens may be performed using a Synteni microarray (Palo Alto, CA) according to the manufacturer's instructions (and essentially as described by Schena et al., *Proc. Natl. Acad. Sci. USA* 93:10614-10619, 1996 and Heller et al., *Proc. Natl. Acad. Sci. USA* 94:2150-2155, 1997). Alternatively, polynucleotides may be amplified from cDNA prepared from cells expressing the proteins described herein, such as colon tumor cells. Such polynucleotides may be amplified via polymerase chain reaction (PCR). For this approach, sequence-specific primers may be designed based on the sequences provided herein, and may be purchased or synthesized.

An amplified portion may be used to isolate a full length gene from a suitable library (*e.g.*, a colon tumor cDNA library) using well known techniques.

Within such techniques, a library (cDNA or genomic) is screened using one or more polynucleotide probes or primers suitable for amplification. Preferably, a library is size-selected to include larger molecules. Random primed libraries may also be preferred for identifying 5' and upstream regions of genes. Genomic libraries are preferred for obtaining introns and extending 5' sequences.

For hybridization techniques, a partial sequence may be labeled (*e.g.*, by nick-translation or end-labeling with ^{32}P) using well known techniques. A bacterial or bacteriophage library is then screened by hybridizing filters containing denatured bacterial colonies (or lawns containing phage plaques) with the labeled probe (*see* Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratories, Cold Spring Harbor, NY, 1989). Hybridizing colonies or plaques are selected and expanded, and the DNA is isolated for further analysis. cDNA clones may be analyzed to determine the amount of additional sequence by, for example, PCR using a primer from the partial sequence and a primer from the vector. Restriction maps and partial sequences may be generated to identify one or more overlapping clones. The complete sequence may then be determined using standard techniques, which may involve generating a series of deletion clones. The resulting overlapping sequences are then assembled into a single contiguous sequence. A full length cDNA molecule can be generated by ligating suitable fragments, using well known techniques.

Alternatively, there are numerous amplification techniques for obtaining a full length coding sequence from a partial cDNA sequence. Within such techniques, amplification is generally performed via PCR. Any of a variety of commercially available kits may be used to perform the amplification step. Primers may be designed using, for example, software well known in the art. Primers are preferably 22-30 nucleotides in length, have a GC content of at least 50% and anneal to the target sequence at temperatures of about 68°C to 72°C. The amplified region may be sequenced as described above, and overlapping sequences assembled into a contiguous sequence.

One such amplification technique is inverse PCR (*see* Triglia et al., *Nucl. Acids Res.* 16:8186, 1988), which uses restriction enzymes to generate a fragment in the known region of the gene. The fragment is then circularized by intramolecular ligation

and used as a template for PCR with divergent primers derived from the known region. Within an alternative approach, sequences adjacent to a partial sequence may be retrieved by amplification with a primer to a linker sequence and a primer specific to a known region. The amplified sequences are typically subjected to a second round of
5 amplification with the same linker primer and a second primer specific to the known region. A variation on this procedure, which employs two primers that initiate extension in opposite directions from the known sequence, is described in WO 96/38591. Another such technique is known as "rapid amplification of cDNA ends" or RACE. This technique involves the use of an internal primer and an external primer,
10 which hybridizes to a polyA region or vector sequence, to identify sequences that are 5' and 3' of a known sequence. Additional techniques include capture PCR (Lagerstrom et al., *PCR Methods Applic.* 1:111-19, 1991) and walking PCR (Parker et al., *Nucl. Acids. Res.* 19:3055-60, 1991). Other methods employing amplification may also be employed to obtain a full length cDNA sequence.

15 In certain instances, it is possible to obtain a full length cDNA sequence by analysis of sequences provided in an expressed sequence tag (EST) database, such as that available from GenBank. Searches for overlapping ESTs may generally be performed using well known programs (e.g., NCBI BLAST searches), and such ESTs may be used to generate a contiguous full length sequence. Full length DNA sequences
20 may also be obtained by analysis of genomic fragments.

Certain nucleic acid sequences of cDNA molecules encoding portions of colon tumor proteins are provided in SEQ ID NOs: 1-1556.

Polynucleotide variants may generally be prepared by any method known in the art, including chemical synthesis by, for example, solid phase
25 phosphoramidite chemical synthesis. Modifications in a polynucleotide sequence may also be introduced using standard mutagenesis techniques, such as oligonucleotide-directed site-specific mutagenesis (see Adelman et al., *DNA* 2:183, 1983). Alternatively, RNA molecules may be generated by *in vitro* or *in vivo* transcription of DNA sequences encoding a colon tumor protein, or portion thereof, provided that the
30 DNA is incorporated into a vector with a suitable RNA polymerase promoter (such as T7 or SP6). Certain portions may be used to prepare an encoded polypeptide, as

described herein. In addition, or alternatively, a portion may be administered to a patient such that the encoded polypeptide is generated *in vivo* (e.g., by transfecting antigen-presenting cells, such as dendritic cells, with a cDNA construct encoding a colon tumor polypeptide, and administering the transfected cells to the patient).

5 A portion of a sequence complementary to a coding sequence (*i.e.*, an antisense polynucleotide) may also be used as a probe or to modulate gene expression. cDNA constructs that can be transcribed into antisense RNA may also be introduced into cells or tissues to facilitate the production of antisense RNA. An antisense polynucleotide may be used, as described herein, to inhibit expression of a tumor
10 protein. Antisense technology can be used to control gene expression through triple-helix formation, which compromises the ability of the double helix to open sufficiently for the binding of polymerases, transcription factors or regulatory molecules (*see* Gee et al., *In Huber and Carr, Molecular and Immunologic Approaches*, Futura Publishing Co. (Mt. Kisco, NY; 1994)). Alternatively, an antisense molecule may be designed to
15 hybridize with a control region of a gene (e.g., promoter, enhancer or transcription initiation site), and block transcription of the gene; or to block translation by inhibiting binding of a transcript to ribosomes.

 A portion of a coding sequence, or of a complementary sequence, may also be designed as a probe or primer to detect gene expression. Probes may be labeled
20 with a variety of reporter groups, such as radionuclides and enzymes, and are preferably at least 10 nucleotides in length, more preferably at least 20 nucleotides in length and still more preferably at least 30 nucleotides in length. Primers, as noted above, are preferably 22-30 nucleotides in length.

 Any polynucleotide may be further modified to increase stability *in vivo*.
25 Possible modifications include, but are not limited to, the addition of flanking sequences at the 5' and/or 3' ends; the use of phosphorothioate or 2' O-methyl rather than phosphodiesterase linkages in the backbone; and/or the inclusion of nontraditional bases such as inosine, queosine and wybutosine, as well as acetyl- methyl-, thio- and other modified forms of adenine, cytidine, guanine, thymine and uridine.

30 Nucleotide sequences as described herein may be joined to a variety of other nucleotide sequences using established recombinant DNA techniques. For

example, a polynucleotide may be cloned into any of a variety of cloning vectors, including plasmids, phagemids, lambda phage derivatives and cosmids. Vectors of particular interest include expression vectors, replication vectors, probe generation vectors and sequencing vectors. In general, a vector will contain an origin of replication functional in at least one organism, convenient restriction endonuclease sites and one or more selectable markers. Other elements will depend upon the desired use, and will be apparent to those of ordinary skill in the art.

Within certain embodiments, polynucleotides may be formulated so as to permit entry into a cell of a mammal, and expression therein. Such formulations are particularly useful for therapeutic purposes, as described below. Those of ordinary skill in the art will appreciate that there are many ways to achieve expression of a polynucleotide in a target cell, and any suitable method may be employed. For example, a polynucleotide may be incorporated into a viral vector such as, but not limited to, adenovirus, adeno-associated virus, retrovirus, or vaccinia or other pox virus (e.g., avian pox virus). The polynucleotides may also be administered as naked plasmid vectors. Techniques for incorporating DNA into such vectors are well known to those of ordinary skill in the art. A retroviral vector may additionally transfer or incorporate a gene for a selectable marker (to aid in the identification or selection of transduced cells) and/or a targeting moiety, such as a gene that encodes a ligand for a receptor on a specific target cell, to render the vector target specific. Targeting may also be accomplished using an antibody, by methods known to those of ordinary skill in the art.

Other formulations for therapeutic purposes include colloidal dispersion systems, such as macromolecule complexes, nanocapsules, microspheres, beads, and lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes. A preferred colloidal system for use as a delivery vehicle *in vitro* and *in vivo* is a liposome (*i.e.*, an artificial membrane vesicle). The preparation and use of such systems is well known in the art.

COLON TUMOR POLYPEPTIDES

Within the context of the present invention, polypeptides may comprise at least an immunogenic portion of a colon tumor protein or a variant thereof, as

described herein. As noted above, a "colon tumor protein" is a protein that is expressed by colon tumor cells. Proteins that are colon tumor proteins also react detectably within an immunoassay (such as an ELISA) with antisera from a patient with colon cancer. Polypeptides as described herein may be of any length. Additional sequences derived
5 from the native protein and/or heterologous sequences may be present, and such sequences may (but need not) possess further immunogenic or antigenic properties.

An "immunogenic portion," as used herein is a portion of a protein that is recognized (*i.e.*, specifically bound) by a B-cell and/or T-cell surface antigen receptor. Such immunogenic portions generally comprise at least 5 amino acid
10 residues, more preferably at least 10, and still more preferably at least 20 amino acid residues of a colon tumor protein or a variant thereof. Certain preferred immunogenic portions include peptides in which an N-terminal leader sequence and/or transmembrane domain have been deleted. Other preferred immunogenic portions may contain a small N- and/or C-terminal deletion (*e.g.*, 1-30 amino acids, preferably 5-15
15 amino acids), relative to the mature protein.

Immunogenic portions may generally be identified using well known techniques, such as those summarized in Paul, *Fundamental Immunology*, 3rd ed., 243-247 (Raven Press, 1993) and references cited therein. Such techniques include screening polypeptides for the ability to react with antigen-specific antibodies, antisera
20 and/or T-cell lines or clones. As used herein, antisera and antibodies are "antigen-specific" if they specifically bind to an antigen (*i.e.*, they react with the protein in an ELISA or other immunoassay, and do not react detectably with unrelated proteins). Such antisera and antibodies may be prepared as described herein, and using well known techniques. An immunogenic portion of a native colon tumor protein is a
25 portion that reacts with such antisera and/or T-cells at a level that is not substantially less than the reactivity of the full length polypeptide (*e.g.*, in an ELISA and/or T-cell reactivity assay). Such immunogenic portions may react within such assays at a level that is similar to or greater than the reactivity of the full length polypeptide. Such screens may generally be performed using methods well known to those of ordinary
30 skill in the art, such as those described in Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, 1988. For example, a polypeptide may be

immobilized on a solid support and contacted with patient sera to allow binding of antibodies within the sera to the immobilized polypeptide. Unbound sera may then be removed and bound antibodies detected using, for example, ^{125}I -labeled Protein A.

As noted above, a composition may comprise a variant of a native colon tumor protein. A polypeptide "variant," as used herein, is a polypeptide that differs from a native colon tumor protein in one or more substitutions, deletions, additions and/or insertions, such that the immunogenicity of the polypeptide is not substantially diminished. In other words, the ability of a variant to react with antigen-specific antisera may be enhanced or unchanged, relative to the native protein, or may be diminished by less than 50%, and preferably less than 20%, relative to the native protein. Such variants may generally be identified by modifying one of the above polypeptide sequences and evaluating the reactivity of the modified polypeptide with antigen-specific antibodies or antisera as described herein. Preferred variants include those in which one or more portions, such as an N-terminal leader sequence or transmembrane domain, have been removed. Other preferred variants include variants in which a small portion (e.g., 1-30 amino acids, preferably 5-15 amino acids) has been removed from the N- and/or C-terminal of the mature protein.

Polypeptide variants preferably exhibit at least about 70%, more preferably at least about 90% and most preferably at least about 95% identity (determined as described above) to the identified polypeptides.

Preferably, a variant contains conservative substitutions. A "conservative substitution" is one in which an amino acid is substituted for another amino acid that has similar properties, such that one skilled in the art of peptide chemistry would expect the secondary structure and hydrophobic nature of the polypeptide to be substantially unchanged. Amino acid substitutions may generally be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity and/or the amphipathic nature of the residues. For example, negatively charged amino acids include aspartic acid and glutamic acid; positively charged amino acids include lysine and arginine; and amino acids with uncharged polar head groups having similar hydrophilicity values include leucine, isoleucine and valine; glycine and alanine; asparagine and glutamine; and serine, threonine, phenylalanine and tyrosine.

Other groups of amino acids that may represent conservative changes include: (1) ala, pro, gly, glu, asp, gln, asn, ser, thr; (2) cys, ser, tyr, thr; (3) val, ile, leu, met, ala, phe; (4) lys, arg, his; and (5) phe, tyr, trp, his. A variant may also, or alternatively, contain nonconservative changes. In a preferred embodiment, variant polypeptides differ from
5 a native sequence by substitution, deletion or addition of five amino acids or fewer. Variants may also (or alternatively) be modified by, for example, the deletion or addition of amino acids that have minimal influence on the immunogenicity, secondary structure and hydrophobic nature of the polypeptide.

As noted above, polypeptides may comprise a signal (or leader)
10 sequence at the N-terminal end of the protein, which co-translationally or post-translationally directs transfer of the protein. The polypeptide may also be conjugated to a linker or other sequence for ease of synthesis, purification or identification of the polypeptide (*e.g.*, poly-His), or to enhance binding of the polypeptide to a solid support. For example, a polypeptide may be conjugated to an immunoglobulin Fc region.

15 Polypeptides may be prepared using any of a variety of well known techniques. Recombinant polypeptides encoded by DNA sequences as described above may be readily prepared from the DNA sequences using any of a variety of expression vectors known to those of ordinary skill in the art. Expression may be achieved in any appropriate host cell that has been transformed or transfected with an expression vector
20 containing a DNA molecule that encodes a recombinant polypeptide. Suitable host cells include prokaryotes, yeast, and higher eukaryotic cells, such as mammalian cells and plant cells. Preferably, the host cells employed are *E. coli*, yeast or a mammalian cell line such as COS or CHO. Supernatants from suitable host/vector systems which secrete recombinant protein or polypeptide into culture media may be first concentrated

polypeptides may be synthesized using any of the commercially available solid-phase techniques, such as the Merrifield solid-phase synthesis method, where amino acids are sequentially added to a growing amino acid chain. *See Merrifield, J. Am. Chem. Soc.* 85:2149-2146, 1963. Equipment for automated synthesis of polypeptides is
5 commercially available from suppliers such as Perkin Elmer/Applied BioSystems Division (Foster City, CA), and may be operated according to the manufacturer's instructions.

Within certain specific embodiments, a polypeptide may be a fusion protein that comprises multiple polypeptides as described herein, or that comprises at
10 least one polypeptide as described herein and an unrelated sequence, such as a known tumor protein. A fusion partner may, for example, assist in providing T helper epitopes (an immunological fusion partner), preferably T helper epitopes recognized by humans, or may assist in expressing the protein (an expression enhancer) at higher yields than the native recombinant protein. Certain preferred fusion partners are both
15 immunological and expression enhancing fusion partners. Other fusion partners may be selected so as to increase the solubility of the protein or to enable the protein to be targeted to desired intracellular compartments. Still further fusion partners include affinity tags, which facilitate purification of the protein.

Fusion proteins may generally be prepared using standard techniques,
20 including chemical conjugation. Preferably, a fusion protein is expressed as a recombinant protein, allowing the production of increased levels, relative to a non-fused protein, in an expression system. Briefly, DNA sequences encoding the polypeptide components may be assembled separately, and ligated into an appropriate expression vector. The 3' end of the DNA sequence encoding one polypeptide component is
25 ligated, with or without a peptide linker, to the 5' end of a DNA sequence encoding the second polypeptide component so that the reading frames of the sequences are in phase. This permits translation into a single fusion protein that retains the biological activity of both component polypeptides.

A peptide linker sequence may be employed to separate the first and
30 second polypeptide components by a distance sufficient to ensure that each polypeptide folds into its secondary and tertiary structures. Such a peptide linker sequence is

incorporated into the fusion protein using standard techniques well known in the art. Suitable peptide linker sequences may be chosen based on the following factors: (1) their ability to adopt a flexible extended conformation; (2) their inability to adopt a secondary structure that could interact with functional epitopes on the first and second polypeptides; and (3) the lack of hydrophobic or charged residues that might react with the polypeptide functional epitopes. Preferred peptide linker sequences contain Gly, Asn and Ser residues. Other near neutral amino acids, such as Thr and Ala may also be used in the linker sequence. Amino acid sequences which may be usefully employed as linkers include those disclosed in Maratea et al., *Gene* 40:39-46, 1985; Murphy et al., *Proc. Natl. Acad. Sci. USA* 83:8258-8262, 1986; U.S. Patent No. 4,935,233 and U.S. Patent No. 4,751,180. The linker sequence may generally be from 1 to about 50 amino acids in length. Linker sequences are not required when the first and second polypeptides have non-essential N-terminal amino acid regions that can be used to separate the functional domains and prevent steric interference.

The ligated DNA sequences are operably linked to suitable transcriptional or translational regulatory elements. The regulatory elements responsible for expression of DNA are located only 5' to the DNA sequence encoding the first polypeptides. Similarly, stop codons required to end translation and transcription termination signals are only present 3' to the DNA sequence encoding the second polypeptide.

Fusion proteins are also provided. Such proteins comprise a polypeptide as described herein together with an unrelated immunogenic protein. Preferably the immunogenic protein is capable of eliciting a recall response. Examples of such proteins include tetanus, tuberculosis and hepatitis proteins (*see*, for example, Stoute et al. *New Engl. J. Med.*, 336:86-91, 1997).

Within preferred embodiments, an immunological fusion partner is derived from protein D, a surface protein of the gram-negative bacterium *Haemophilus influenza B* (WO 91/18926). Preferably, a protein D derivative comprises approximately the first third of the protein (*e.g.*, the first N-terminal 100-110 amino acids), and a protein D derivative may be lipidated. Within certain preferred embodiments, the first 109 residues of a Lipoprotein D fusion partner is included on the

N-terminus to provide the polypeptide with additional exogenous T-cell epitopes and to increase the expression level in *E. coli* (thus functioning as an expression enhancer). The lipid tail ensures optimal presentation of the antigen to antigen presenting cells. Other fusion partners include the non-structural protein from influenzae virus, NS1 (hemagglutinin). Typically, the N-terminal 81 amino acids are used, although different fragments that include T-helper epitopes may be used.

In another embodiment, the immunological fusion partner is the protein known as LYTA, or a portion thereof (preferably a C-terminal portion). LYTA is derived from *Streptococcus pneumoniae*, which synthesizes an N-acetyl-L-alanine amidase known as amidase LYTA (encoded by the *LytA* gene; *Gene* 43:265-292, 1986). LYTA is an autolysin that specifically degrades certain bonds in the peptidoglycan backbone. The C-terminal domain of the LYTA protein is responsible for the affinity to the choline or to some choline analogues such as DEAE. This property has been exploited for the development of *E. coli* C-LYTA expressing plasmids useful for expression of fusion proteins. Purification of hybrid proteins containing the C-LYTA fragment at the amino terminus has been described (see *Biotechnology* 10:795-798, 1992). Within a preferred embodiment, a repeat portion of LYTA may be incorporated into a fusion protein. A repeat portion is found in the C-terminal region starting at residue 178. A particularly preferred repeat portion incorporates residues 188-305.

In general, polypeptides (including fusion proteins) and polynucleotides as described herein are isolated. An "isolated" polypeptide or polynucleotide is one that is removed from its original environment. For example, a naturally-occurring protein is isolated if it is separated from some or all of the coexisting materials in the natural system. Preferably, such polypeptides are at least about 90% pure, more preferably at least about 95% pure and most preferably at least about 99% pure. A polynucleotide is considered to be isolated if, for example, it is cloned into a vector that is not a part of the natural environment.

BINDING AGENTS

The present invention further provides agents, such as antibodies and antigen-binding fragments thereof, that specifically bind to a colon tumor protein. As used herein, an antibody, or antigen-binding fragment thereof, is said to "specifically bind" to a colon tumor protein if it reacts at a detectable level (within, for example, an ELISA) with a colon tumor protein, and does not react detectably with unrelated proteins under similar conditions. As used herein, "binding" refers to a noncovalent association between two separate molecules such that a complex is formed. The ability to bind may be evaluated by, for example, determining a binding constant for the formation of the complex. The binding constant is the value obtained when the concentration of the complex is divided by the product of the component concentrations. In general, two compounds are said to "bind," in the context of the present invention, when the binding constant for complex formation exceeds about 10^3 L/mol. The binding constant may be determined using methods well known in the art.

Binding agents may be further capable of differentiating between patients with and without a cancer, such as colon cancer, using the representative assays provided herein. In other words, antibodies or other binding agents that bind to a colon tumor protein will generate a signal indicating the presence of a cancer in at least about 20% of patients with the disease, and will generate a negative signal indicating the absence of the disease in at least about 90% of individuals without the cancer. To determine whether a binding agent satisfies this requirement, biological samples (*e.g.*, blood, sera, sputum, urine and/or tumor biopsies) from patients with and without a cancer (as determined using standard clinical tests) may be assayed as described herein for the presence of polypeptides that bind to the binding agent. It will be apparent that a statistically significant number of samples with and without the disease should be assayed. Each binding agent should satisfy the above criteria; however, those of ordinary skill in the art will recognize that binding agents may be used in combination to improve sensitivity.

Any agent that satisfies the above requirements may be a binding agent. For example, a binding agent may be a ribosome, with or without a peptide component, an RNA molecule or a polypeptide. In a preferred embodiment, a binding agent is an

antibody or an antigen-binding fragment thereof. Antibodies may be prepared by any of a variety of techniques known to those of ordinary skill in the art. *See, e.g.,* Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, 1988. In general, antibodies can be produced by cell culture techniques, including the generation
5 of monoclonal antibodies as described herein, or via transfection of antibody genes into suitable bacterial or mammalian cell hosts, in order to allow for the production of recombinant antibodies. In one technique, an immunogen comprising the polypeptide is initially injected into any of a wide variety of mammals (*e.g.,* mice, rats, rabbits, sheep or goats). In this step, the polypeptides of this invention may serve as the
10 immunogen without modification. Alternatively, particularly for relatively short polypeptides, a superior immune response may be elicited if the polypeptide is joined to a carrier protein, such as bovine serum albumin or keyhole limpet hemocyanin. The immunogen is injected into the animal host, preferably according to a predetermined schedule incorporating one or more booster immunizations, and the animals are bled
15 periodically. Polyclonal antibodies specific for the polypeptide may then be purified from such antisera by, for example, affinity chromatography using the polypeptide coupled to a suitable solid support.

Monoclonal antibodies specific for an antigenic polypeptide of interest may be prepared, for example, using the technique of Kohler and Milstein, *Eur. J.*
20 *Immunol.* 6:511-519, 1976, and improvements thereto. Briefly, these methods involve the preparation of immortal cell lines capable of producing antibodies having the desired specificity (*i.e.,* reactivity with the polypeptide of interest). Such cell lines may be produced, for example, from spleen cells obtained from an animal immunized as described above. The spleen cells are then immortalized by, for example, fusion with a
25 myeloma cell fusion partner, preferably one that is syngeneic with the immunized animal. A variety of fusion techniques may be employed. For example, the spleen cells and myeloma cells may be combined with a nonionic detergent for a few minutes and then plated at low density on a selective medium that supports the growth of hybrid cells, but not myeloma cells. A preferred selection technique uses HAT (hypoxanthine,
30 aminopterin, thymidine) selection. After a sufficient time, usually about 1 to 2 weeks, colonies of hybrids are observed. Single colonies are selected and their culture

supernatants tested for binding activity against the polypeptide. Hybridomas having high reactivity and specificity are preferred.

Monoclonal antibodies may be isolated from the supernatants of growing hybridoma colonies. In addition, various techniques may be employed to enhance the yield, such as injection of the hybridoma cell line into the peritoneal cavity of a suitable vertebrate host, such as a mouse. Monoclonal antibodies may then be harvested from the ascites fluid or the blood. Contaminants may be removed from the antibodies by conventional techniques, such as chromatography, gel filtration, precipitation, and extraction. The polypeptides of this invention may be used in the purification process in, for example, an affinity chromatography step.

Within certain embodiments, the use of antigen-binding fragments of antibodies may be preferred. Such fragments include Fab fragments, which may be prepared using standard techniques. Briefly, immunoglobulins may be purified from rabbit serum by affinity chromatography on Protein A bead columns (Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, 1988) and digested by papain to yield Fab and Fc fragments. The Fab and Fc fragments may be separated by affinity chromatography on protein A bead columns.

Monoclonal antibodies of the present invention may be coupled to one or more therapeutic agents. Suitable agents in this regard include radionuclides, differentiation inducers, drugs, toxins, and derivatives thereof. Preferred radionuclides include ^{90}Y , ^{123}I , ^{125}I , ^{131}I , ^{186}Re , ^{188}Re , ^{211}At , and ^{212}Bi . Preferred drugs include methotrexate, and pyrimidine and purine analogs. Preferred differentiation inducers include phorbol esters and butyric acid. Preferred toxins include ricin, abrin, diphtheria toxin, cholera toxin, gelonin, *Pseudomonas* exotoxin, *Shigella* toxin, and pokeweed antiviral protein.

A therapeutic agent may be coupled (*e.g.*, covalently bonded) to a suitable monoclonal antibody either directly or indirectly (*e.g.*, via a linker group). A direct reaction between an agent and an antibody is possible when each possesses a substituent capable of reacting with the other. For example, a nucleophilic group, such as an amino or sulfhydryl group, on one may be capable of reacting with a carbonyl-

containing group, such as an anhydride or an acid halide, or with an alkyl group containing a good leaving group (*e.g.*, a halide) on the other.

Alternatively, it may be desirable to couple a therapeutic agent and an antibody via a linker group. A linker group can function as a spacer to distance an antibody from an agent in order to avoid interference with binding capabilities. A linker group can also serve to increase the chemical reactivity of a substituent on an agent or an antibody, and thus increase the coupling efficiency. An increase in chemical reactivity may also facilitate the use of agents, or functional groups on agents, which otherwise would not be possible.

It will be evident to those skilled in the art that a variety of bifunctional or polyfunctional reagents, both homo- and hetero-functional (such as those described in the catalog of the Pierce Chemical Co., Rockford, IL), may be employed as the linker group. Coupling may be effected, for example, through amino groups, carboxyl groups, sulfhydryl groups or oxidized carbohydrate residues. There are numerous references describing such methodology, *e.g.*, U.S. Patent No. 4,671,958, to Rodwell et al.

Where a therapeutic agent is more potent when free from the antibody portion of the immunoconjugates of the present invention, it may be desirable to use a linker group which is cleavable during or upon internalization into a cell. A number of different cleavable linker groups have been described. The mechanisms for the intracellular release of an agent from these linker groups include cleavage by reduction of a disulfide bond (*e.g.*, U.S. Patent No. 4,489,710, to Spitler), by irradiation of a photolabile bond (*e.g.*, U.S. Patent No. 4,625,014, to Senter et al.), by hydrolysis of derivatized amino acid side chains (*e.g.*, U.S. Patent No. 4,638,045, to Kohn et al.), by serum complement-mediated hydrolysis (*e.g.*, U.S. Patent No. 4,671,958, to Rodwell et al.), and acid-catalyzed hydrolysis (*e.g.*, U.S. Patent No. 4,569,789, to Blattler et al.).

It may be desirable to couple more than one agent to an antibody. In one embodiment, multiple molecules of an agent are coupled to one antibody molecule. In another embodiment, more than one type of agent may be coupled to one antibody. Regardless of the particular embodiment, immunoconjugates with more than one agent may be prepared in a variety of ways. For example, more than one agent may be

coupled directly to an antibody molecule, or linkers that provide multiple sites for attachment can be used. Alternatively, a carrier can be used.

A carrier may bear the agents in a variety of ways, including covalent bonding either directly or via a linker group. Suitable carriers include proteins such as albumins (*e.g.*, U.S. Patent No. 4,507,234, to Kato et al.), peptides and polysaccharides such as aminodextran (*e.g.*, U.S. Patent No. 4,699,784, to Shih et al.). A carrier may also bear an agent by noncovalent bonding or by encapsulation, such as within a liposome vesicle (*e.g.*, U.S. Patent Nos. 4,429,008 and 4,873,088). Carriers specific for radionuclide agents include radiohalogenated small molecules and chelating compounds. For example, U.S. Patent No. 4,735,792 discloses representative radiohalogenated small molecules and their synthesis. A radionuclide chelate may be formed from chelating compounds that include those containing nitrogen and sulfur atoms as the donor atoms for binding the metal, or metal oxide, radionuclide. For example, U.S. Patent No. 4,673,562, to Davison et al. discloses representative chelating compounds and their synthesis.

A variety of routes of administration for the antibodies and immunoconjugates may be used. Typically, administration will be intravenous, intramuscular, subcutaneous or in the bed of a resected tumor. It will be evident that the precise dose of the antibody/immunoconjugate will vary depending upon the antibody used, the antigen density on the tumor, and the rate of clearance of the antibody.

T CELLS

Immunotherapeutic compositions may also, or alternatively, comprise T cells specific for a colon tumor protein. Such cells may generally be prepared *in vitro* or *ex vivo*, using standard procedures. For example, T cells may be isolated from bone marrow, peripheral blood, or a fraction of bone marrow or peripheral blood of a patient, using a commercially available cell separation system, such as the Isolex™ System, available from Nexell Therapeutics, Inc. (Irvine, CA; see also U.S. Patent No. 5,240,856; U.S. Patent No. 5,215,926; WO 89/06280; WO 91/16116 and WO

92/07243). Alternatively, T cells may be derived from related or unrelated humans, non-human mammals, cell lines or cultures.

T cells may be stimulated with a colon tumor polypeptide, polynucleotide encoding a colon tumor polypeptide and/or an antigen presenting cell (APC) that expresses such a polypeptide. Such stimulation is performed under conditions and for a time sufficient to permit the generation of T cells that are specific for the polypeptide. Preferably, a colon tumor polypeptide or polynucleotide is present within a delivery vehicle, such as a microsphere, to facilitate the generation of specific T cells.

T cells are considered to be specific for a colon tumor polypeptide if the T cells specifically proliferate, secrete cytokines or kill target cells coated with the polypeptide or expressing a gene encoding the polypeptide. T cell specificity may be evaluated using any of a variety of standard techniques. For example, within a chromium release assay or proliferation assay, a stimulation index of more than two fold increase in lysis and/or proliferation, compared to negative controls, indicates T cell specificity. Such assays may be performed, for example, as described in Chen et al., *Cancer Res.* 54:1065-1070, 1994. Alternatively, detection of the proliferation of T cells may be accomplished by a variety of known techniques. For example, T cell proliferation can be detected by measuring an increased rate of DNA synthesis (e.g., by pulse-labeling cultures of T cells with tritiated thymidine and measuring the amount of tritiated thymidine incorporated into DNA). Contact with a colon tumor polypeptide (100 ng/ml - 100 µg/ml, preferably 200 ng/ml - 25 µg/ml) for 3 - 7 days should result in at least a two fold increase in proliferation of the T cells. Contact as described above for 2-3 hours should result in activation of the T cells, as measured using standard cytokine assays in which a two fold increase in the level of cytokine release (e.g., TNF or IFN-γ) is indicative of T cell activation (see Coligan et al., *Current Protocols in Immunology*, vol. 1, Wiley Interscience (Greene 1998)). T cells that have been activated in response to a colon tumor polypeptide, polynucleotide or polypeptide-expressing APC may be CD4⁺ and/or CD8⁺. Colon tumor protein-specific T cells may be expanded using standard techniques. Within preferred embodiments, the T cells are

derived from a patient, a related donor or an unrelated donor, and are administered to the patient following stimulation and expansion.

For therapeutic purposes, CD4⁺ or CD8⁺ T cells that proliferate in response to a colon tumor polypeptide, polynucleotide or APC can be expanded in number either *in vitro* or *in vivo*. Proliferation of such T cells *in vitro* may be accomplished in a variety of ways. For example, the T cells can be re-exposed to a colon tumor polypeptide, or a short peptide corresponding to an immunogenic portion of such a polypeptide, with or without the addition of T cell growth factors, such as interleukin-2, and/or stimulator cells that synthesize a colon tumor polypeptide. Alternatively, one or more T cells that proliferate in the presence of a colon tumor protein can be expanded in number by cloning. Methods for cloning cells are well known in the art, and include limiting dilution.

PHARMACEUTICAL COMPOSITIONS AND VACCINES

Within certain aspects, polypeptides, polynucleotides, T cells and/or binding agents described herein may be incorporated into pharmaceutical compositions or immunogenic compositions (*i.e.*, vaccines). Pharmaceutical compositions comprise one or more such compounds and a physiologically acceptable carrier. Vaccines may comprise one or more such compounds and an immunostimulant. An immunostimulant may be any substance that enhances or potentiates an immune response (antibody and/or cell-mediated) to an exogenous antigen. Examples of immunostimulants include adjuvants, biodegradable microspheres (*e.g.*, polylactic galactide) and liposomes (into which the compound is incorporated; *see e.g.*, Fullerton, U.S. Patent No. 4,235,877). Vaccine preparation is generally described in, for example, M.F. Powell and M.J. Newman, eds., "Vaccine Design (the subunit and adjuvant approach)," Plenum Press (NY, 1995). Pharmaceutical compositions and vaccines within the scope of the present invention may also contain other compounds, which may be biologically active or inactive. For example, one or more immunogenic portions of other tumor antigens may be present, either incorporated into a fusion polypeptide or as a separate compound, within the composition or vaccine.

A pharmaceutical composition or vaccine may contain DNA encoding one or more of the polypeptides as described above, such that the polypeptide is generated *in situ*. As noted above, the DNA may be present within any of a variety of delivery systems known to those of ordinary skill in the art, including nucleic acid expression systems, bacteria and viral expression systems. Numerous gene delivery techniques are well known in the art, such as those described by Rolland, *Crit. Rev. Therap. Drug Carrier Systems* 15:143-198, 1998, and references cited therein. Appropriate nucleic acid expression systems contain the necessary DNA sequences for expression in the patient (such as a suitable promoter and terminating signal). Bacterial delivery systems involve the administration of a bacterium (such as *Bacillus-Calmette-Guerrin*) that expresses an immunogenic portion of the polypeptide on its cell surface or secretes such an epitope. In a preferred embodiment, the DNA may be introduced using a viral expression system (e.g., vaccinia or other pox virus, retrovirus, or adenovirus), which may involve the use of a non-pathogenic (defective), replication competent virus. Suitable systems are disclosed, for example, in Fisher-Hoch et al., *Proc. Natl. Acad. Sci. USA* 86:317-321, 1989; Flexner et al., *Ann. N.Y. Acad. Sci.* 569:86-103, 1989; Flexner et al., *Vaccine* 8:17-21, 1990; U.S. Patent Nos. 4,603,112, 4,769,330, and 5,017,487; WO 89/01973; U.S. Patent No. 4,777,127; GB 2,200,651; EP 0,345,242; WO 91/02805; Berkner, *Biotechniques* 6:616-627, 1988; Rosenfeld et al., *Science* 252:431-434, 1991; Kolls et al., *Proc. Natl. Acad. Sci. USA* 91:215-219, 1994; Kass-Eisler et al., *Proc. Natl. Acad. Sci. USA* 90:11498-11502, 1993; Guzman et al., *Circulation* 88:2838-2848, 1993; and Guzman et al., *Cir. Res.* 73:1202-1207, 1993. Techniques for incorporating DNA into such expression systems are well known to those of ordinary skill in the art. The DNA may also be "naked," as described, for example, in Ulmer et al., *Science* 259:1745-1749, 1993 and reviewed by Cohen, *Science* 259:1691-1692, 1993. The uptake of naked DNA may be increased by coating the DNA onto biodegradable beads, which are efficiently transported into the cells. It will be apparent that a vaccine may comprise both a polynucleotide and a polypeptide component. Such vaccines may provide for an enhanced immune response.

It will be apparent that a vaccine may contain pharmaceutically acceptable salts of the polynucleotides and polypeptides provided herein. Such salts

may be prepared from pharmaceutically acceptable non-toxic bases, including organic bases (*e.g.*, salts of primary, secondary and tertiary amines and basic amino acids) and inorganic bases (*e.g.*, sodium, potassium, lithium, ammonium, calcium and magnesium salts).

5 While any suitable carrier known to those of ordinary skill in the art may be employed in the pharmaceutical compositions of this invention, the type of carrier will vary depending on the mode of administration. Compositions of the present invention may be formulated for any appropriate manner of administration, including for example, topical, oral, nasal, intravenous, intracranial, intraperitoneal, subcutaneous
10 or intramuscular administration. For parenteral administration, such as subcutaneous injection, the carrier preferably comprises water, saline, alcohol, a fat, a wax or a buffer. For oral administration, any of the above carriers or a solid carrier, such as mannitol, lactose, starch, magnesium stearate, sodium saccharine, talcum, cellulose, glucose, sucrose, and magnesium carbonate, may be employed. Biodegradable microspheres
15 (*e.g.*, polylactate polyglycolate) may also be employed as carriers for the pharmaceutical compositions of this invention. Suitable biodegradable microspheres are disclosed, for example, in U.S. Patent Nos. 4,897,268; 5,075,109; 5,928,647; 5,811,128; 5,820,883; 5,853,763; 5,814,344 and 5,942,252.

Such compositions may also comprise buffers (*e.g.*, neutral buffered
20 saline or phosphate buffered saline), carbohydrates (*e.g.*, glucose, mannose, sucrose or dextrans), mannitol, proteins, polypeptides or amino acids such as glycine, antioxidants, bacteriostats, chelating agents such as EDTA or glutathione, adjuvants (*e.g.*, aluminum hydroxide), solutes that render the formulation isotonic, hypotonic or weakly hypertonic with the blood of a recipient, suspending agents, thickening agents and/or preservatives.
25 Alternatively, compositions of the present invention may be formulated as a lyophilizate. Compounds may also be encapsulated within liposomes using well known technology.

Any of a variety of immunostimulants may be employed in the vaccines of this invention. For example, an adjuvant may be included. Most adjuvants contain a
30 substance designed to protect the antigen from rapid catabolism, such as aluminum hydroxide or mineral oil, and a stimulator of immune responses, such as lipid A,

Bordetella pertussis or *Mycobacterium tuberculosis* derived proteins. Suitable adjuvants are commercially available as, for example, Freund's Incomplete Adjuvant and Complete Adjuvant (Difco Laboratories, Detroit, MI); Merck Adjuvant 65 (Merck and Company, Inc., Rahway, NJ); AS-2 (SmithKline Beecham, Philadelphia, PA);
5 aluminum salts such as aluminum hydroxide gel (alum) or aluminum phosphate; salts of calcium, iron or zinc; an insoluble suspension of acylated tyrosine; acylated sugars; cationically or anionically derivatized polysaccharides; polyphosphazenes; biodegradable microspheres; monophosphoryl lipid A and quil A. Cytokines, such as GM-CSF or interleukin-2, -7, or -12, may also be used as adjuvants.

10 Within the vaccines provided herein, the adjuvant composition is preferably designed to induce an immune response predominantly of the Th1 type. High levels of Th1-type cytokines (e.g., IFN- γ , TNF α , IL-2 and IL-12) tend to favor the induction of cell mediated immune responses to an administered antigen. In contrast, high levels of Th2-type cytokines (e.g., IL-4, IL-5, IL-6 and IL-10) tend to favor the
15 induction of humoral immune responses. Following application of a vaccine as provided herein, a patient will support an immune response that includes Th1- and Th2-type responses. Within a preferred embodiment, in which a response is predominantly Th1-type, the level of Th1-type cytokines will increase to a greater extent than the level of Th2-type cytokines. The levels of these cytokines may be readily assessed using
20 standard assays. For a review of the families of cytokines, see Mosmann and Coffman, *Ann. Rev. Immunol.* 7:145-173, 1989.

Preferred adjuvants for use in eliciting a predominantly Th1-type response include, for example, a combination of monophosphoryl lipid A, preferably 3-de-O-acylated monophosphoryl lipid A (3D-MPL), together with an aluminum salt.
25 MPL adjuvants are available from Corixa Corporation (Seattle, WA; see US Patent Nos. 4,436,727; 4,877,611; 4,866,034 and 4,912,094). CpG-containing oligonucleotides (in which the CpG dinucleotide is unmethylated) also induce a predominantly Th1 response. Such oligonucleotides are well known and are described, for example, in WO 96/02555 and WO 99/33488. Immunostimulatory DNA sequences
30 are also described, for example, by Sato et al., *Science* 273:352, 1996. Another preferred adjuvant is a saponin, preferably QS21 (Aquila Biopharmaceuticals Inc.,

Framingham, MA), which may be used alone or in combination with other adjuvants. For example, an enhanced system involves the combination of a monophosphoryl lipid A and saponin derivative, such as the combination of QS21 and 3D-MPL as described in WO 94/00153, or a less reactogenic composition where the QS21 is quenched with
5 cholesterol, as described in WO 96/33739. Other preferred formulations comprise an oil-in-water emulsion and tocopherol. A particularly potent adjuvant formulation involving QS21, 3D-MPL and tocopherol in an oil-in-water emulsion is described in WO 95/17210.

Other preferred adjuvants include Montanide ISA 720 (Seppic, France),
10 SAF (Chiron, California, United States), ISCOMS (CSL), MF-59 (Chiron), the SBAS series of adjuvants (e.g., SBAS-2 or SBAS-4, available from SmithKline Beecham, Rixensart, Belgium), Detox (Ribi ImmunoChem Research Inc., Hamilton, MT), RC-529 (Ribi ImmunoChem Research Inc., Hamilton, MT) and Aminoalkyl glucosaminide 4-phosphates (AGPs).

15 Any vaccine provided herein may be prepared using well known methods that result in a combination of antigen, immune response enhancer and a suitable carrier or excipient. The compositions described herein may be administered as part of a sustained release formulation (i.e., a formulation such as a capsule, sponge or gel (composed of polysaccharides, for example) that effects a slow release of
20 compound following administration). Such formulations may generally be prepared using well known technology (see, e.g., Coombes et al., *Vaccine* 14:1429-1438, 1996) and administered by, for example, oral, rectal or subcutaneous implantation, or by implantation at the desired target site. Sustained-release formulations may contain a polypeptide, polynucleotide or antibody dispersed in a carrier matrix and/or contained
25 within a reservoir surrounded by a rate controlling membrane.

Carriers for use within such formulations are biocompatible, and may also be biodegradable; preferably the formulation provides a relatively constant level of active component release. Such carriers include microparticles of poly(lactide-co-glycolide), as well as polyacrylate, latex, starch, cellulose and dextran. Other delayed-
30 release carriers include supramolecular biovectors, which comprise a non-liquid hydrophilic core (e.g., a cross-linked polysaccharide or oligosaccharide) and,

optionally, an external layer comprising an amphiphilic compound, such as a phospholipid (*see e.g.*, U.S. Patent No. 5,151,254 and PCT applications WO 94/20078, WO/94/23701 and WO 96/06638). The amount of active compound contained within a sustained release formulation depends upon the site of implantation, the rate and
5 expected duration of release and the nature of the condition to be treated or prevented.

Any of a variety of delivery vehicles may be employed within pharmaceutical compositions and vaccines to facilitate production of an antigen-specific immune response that targets tumor cells. Delivery vehicles include antigen presenting cells (APCs), such as dendritic cells, macrophages, B cells, monocytes and
10 other cells that may be engineered to be efficient APCs. Such cells may, but need not, be genetically modified to increase the capacity for presenting the antigen, to improve activation and/or maintenance of the T cell response, to have anti-tumor effects *per se* and/or to be immunologically compatible with the receiver (*i.e.*, matched HLA haplotype). APCs may generally be isolated from any of a variety of biological fluids
15 and organs, including tumor and peritumoral tissues, and may be autologous, allogeneic, syngeneic or xenogeneic cells.

Certain preferred embodiments of the present invention use dendritic cells or progenitors thereof as antigen-presenting cells. Dendritic cells are highly potent APCs (Banchereau and Steinman, *Nature* 392:245-251, 1998) and have been shown to
20 be effective as a physiological adjuvant for eliciting prophylactic or therapeutic antitumor immunity (*see* Timmerman and Levy, *Ann. Rev. Med.* 50:507-529, 1999). In general, dendritic cells may be identified based on their typical shape (*stellate in situ*, with marked cytoplasmic processes (*dendrites*) visible *in vitro*), their ability to take up, process and present antigens with high efficiency and their ability to activate naïve T
25 cell responses. Dendritic cells may, of course, be engineered to express specific cell-surface receptors or ligands that are not commonly found on dendritic cells *in vivo* or *ex vivo*, and such modified dendritic cells are contemplated by the present invention. As an alternative to dendritic cells, secreted vesicles antigen-loaded dendritic cells (called exosomes) may be used within a vaccine (*see* Zitvogel et al., *Nature Med.* 4:594-600,
30 1998).

Dendritic cells and progenitors may be obtained from peripheral blood, bone marrow, tumor-infiltrating cells, peritumoral tissues-infiltrating cells, lymph nodes, spleen, skin, umbilical cord blood or any other suitable tissue or fluid. For example, dendritic cells may be differentiated *ex vivo* by adding a combination of
5 cytokines such as GM-CSF, IL-4, IL-13 and/or TNF α to cultures of monocytes harvested from peripheral blood. Alternatively, CD34 positive cells harvested from peripheral blood, umbilical cord blood or bone marrow may be differentiated into dendritic cells by adding to the culture medium combinations of GM-CSF, IL-3, TNF α , CD40 ligand, LPS, flt3 ligand and/or other compound(s) that induce differentiation,
10 maturation and proliferation of dendritic cells.

Dendritic cells are conveniently categorized as "immature" and "mature" cells, which allows a simple way to discriminate between two well characterized phenotypes. However, this nomenclature should not be construed to exclude all possible intermediate stages of differentiation. Immature dendritic cells are
15 characterized as APC with a high capacity for antigen uptake and processing, which correlates with the high expression of Fc γ receptor and mannose receptor. The mature phenotype is typically characterized by a lower expression of these markers, but a high expression of cell surface molecules responsible for T cell activation such as class I and class II MHC, adhesion molecules (*e.g.*, CD54 and CD11) and costimulatory molecules
20 (*e.g.*, CD40, CD80, CD86 and 4-1BB).

APCs may generally be transfected with a polynucleotide encoding a colon tumor protein (or portion or other variant thereof) such that the colon tumor polypeptide, or an immunogenic portion thereof, is expressed on the cell surface. Such transfection may take place *ex vivo*, and a composition or vaccine comprising such
25 transfected cells may then be used for therapeutic purposes, as described herein. Alternatively, a gene delivery vehicle that targets a dendritic or other antigen presenting cell may be administered to a patient, resulting in transfection that occurs *in vivo*. *In vivo* and *ex vivo* transfection of dendritic cells, for example, may generally be performed using any methods known in the art, such as those described in WO
30 97/24447, or the gene gun approach described by Mahvi et al., *Immunology and cell Biology* 75:456-460, 1997. Antigen loading of dendritic cells may be achieved by

incubating dendritic cells or progenitor cells with the colon tumor polypeptide, DNA (naked or within a plasmid vector) or RNA; or with antigen-expressing recombinant bacterium or viruses (e.g., vaccinia, fowlpox, adenovirus or lentivirus vectors). Prior to loading, the polypeptide may be covalently conjugated to an immunological partner that provides T cell help (e.g., a carrier molecule). Alternatively, a dendritic cell may be pulsed with a non-conjugated immunological partner, separately or in the presence of the polypeptide.

Vaccines and pharmaceutical compositions may be presented in unit-dose or multi-dose containers, such as sealed ampoules or vials. Such containers are preferably hermetically sealed to preserve sterility of the formulation until use. In general, formulations may be stored as suspensions, solutions or emulsions in oily or aqueous vehicles. Alternatively, a vaccine or pharmaceutical composition may be stored in a freeze-dried condition requiring only the addition of a sterile liquid carrier immediately prior to use.

15 CANCER THERAPY

In further aspects of the present invention, the compositions described herein may be used for immunotherapy of cancer, such as colon cancer. Within such methods, pharmaceutical compositions and vaccines are typically administered to a patient. As used herein, a "patient" refers to any warm-blooded animal, preferably a human. A patient may or may not be afflicted with cancer. Accordingly, the above pharmaceutical compositions and vaccines may be used to prevent the development of a cancer or to treat a patient afflicted with a cancer. A cancer may be diagnosed using criteria generally accepted in the art, including the presence of a malignant tumor. Pharmaceutical compositions and vaccines may be administered either prior to or following surgical removal of primary tumors and/or treatment such as administration of radiotherapy or conventional chemotherapeutic drugs. Administration may be by any suitable method, including administration by intravenous, intraperitoneal, intramuscular, subcutaneous, intranasal, intradermal, anal, vaginal, topical and oral routes.

Within certain embodiments, immunotherapy may be active immunotherapy, in which treatment relies on the *in vivo* stimulation of the endogenous host immune system to react against tumors with the administration of immune response-modifying agents (such as polypeptides and polynucleotides as provided
5 herein).

Within other embodiments, immunotherapy may be passive immunotherapy, in which treatment involves the delivery of agents with established tumor-immune reactivity (such as effector cells or antibodies) that can directly or indirectly mediate antitumor effects and does not necessarily depend on an intact host
10 immune system. Examples of effector cells include T cells as discussed above, T lymphocytes (such as CD8⁺ cytotoxic T lymphocytes and CD4⁺ T-helper tumor-infiltrating lymphocytes), killer cells (such as Natural Killer cells and lymphokine-activated killer cells), B cells and antigen-presenting cells (such as dendritic cells and macrophages) expressing a polypeptide provided herein. T cell receptors and antibody
15 receptors specific for the polypeptides recited herein may be cloned, expressed and transferred into other vectors or effector cells for adoptive immunotherapy. The polypeptides provided herein may also be used to generate antibodies or anti-idiotypic antibodies (as described above and in U.S. Patent No. 4,918,164) for passive immunotherapy.

20 Effector cells may generally be obtained in sufficient quantities for adoptive immunotherapy by growth *in vitro*, as described herein. Culture conditions for expanding single antigen-specific effector cells to several billion in number with retention of antigen recognition *in vivo* are well known in the art. Such *in vitro* culture conditions typically use intermittent stimulation with antigen, often in the presence of
25 cytokines (such as IL-2) and non-dividing feeder cells. As noted above, immunoreactive polypeptides as provided herein may be used to rapidly expand antigen-specific T cell cultures in order to generate a sufficient number of cells for immunotherapy. In particular, antigen-presenting cells, such as dendritic, macrophage, monocyte, fibroblast and/or B cells, may be pulsed with immunoreactive polypeptides
30 or transfected with one or more polynucleotides using standard techniques well known in the art. For example, antigen-presenting cells can be transfected with a

polynucleotide having a promoter appropriate for increasing expression in a recombinant virus or other expression system. Cultured effector cells for use in therapy must be able to grow and distribute widely, and to survive long term *in vivo*. Studies have shown that cultured effector cells can be induced to grow *in vivo* and to survive
5 long term in substantial numbers by repeated stimulation with antigen supplemented with IL-2 (*see, for example, Cheever et al., Immunological Reviews 157:177, 1997*).

Alternatively, a vector expressing a polypeptide recited herein may be introduced into antigen presenting cells taken from a patient and clonally propagated *ex vivo* for transplant back into the same patient. Transfected cells may be reintroduced
10 into the patient using any means known in the art, preferably in sterile form by intravenous, intracavitary, intraperitoneal or intratumor administration.

Routes and frequency of administration of the therapeutic compositions described herein, as well as dosage, will vary from individual to individual, and may be readily established using standard techniques. In general, the pharmaceutical
15 compositions and vaccines may be administered by injection (*e.g., intracutaneous, intramuscular, intravenous or subcutaneous*), intranasally (*e.g., by aspiration*) or orally. Preferably, between 1 and 10 doses may be administered over a 52 week period. Preferably, 6 doses are administered, at intervals of 1 month, and booster vaccinations may be given periodically thereafter. Alternate protocols may be appropriate for
20 individual patients. A suitable dose is an amount of a compound that, when administered as described above, is capable of promoting an anti-tumor immune response, and is at least 10-50% above the basal (*i.e., untreated*) level. Such response can be monitored by measuring the anti-tumor antibodies in a patient or by vaccine-dependent generation of cytolytic effector cells capable of killing the patient's tumor
25 cells *in vitro*. Such vaccines should also be capable of causing an immune response that leads to an improved clinical outcome (*e.g., more frequent remissions, complete or partial or longer disease-free survival*) in vaccinated patients as compared to non-vaccinated patients. In general, for pharmaceutical compositions and vaccines comprising one or more polypeptides, the amount of each polypeptide present in a dose
30 ranges from about 25 µg to 5 mg per kg of host. Suitable dose sizes will vary with the size of the patient, but will typically range from about 0.1 mL to about 5 mL.

In general, an appropriate dosage and treatment regimen provides the active compound(s) in an amount sufficient to provide therapeutic and/or prophylactic benefit. Such a response can be monitored by establishing an improved clinical outcome (e.g., more frequent remissions, complete or partial, or longer disease-free survival) in treated patients as compared to non-treated patients. Increases in preexisting immune responses to a colon tumor protein generally correlate with an improved clinical outcome. Such immune responses may generally be evaluated using standard proliferation, cytotoxicity or cytokine assays, which may be performed using samples obtained from a patient before and after treatment.

10 METHODS FOR DETECTING CANCER

In general, a cancer may be detected in a patient based on the presence of one or more colon tumor proteins and/or polynucleotides encoding such proteins in a biological sample (for example, blood, sera, sputum urine and/or tumor biopsies) obtained from the patient. In other words, such proteins may be used as markers to indicate the presence or absence of a cancer such as colon cancer. In addition, such proteins may be useful for the detection of other cancers. The binding agents provided herein generally permit detection of the level of antigen that binds to the agent in the biological sample. Polynucleotide primers and probes may be used to detect the level of mRNA encoding a tumor protein, which is also indicative of the presence or absence of a cancer. In general, a colon tumor sequence should be present at a level that is at least three fold higher in tumor tissue than in normal tissue

There are a variety of assay formats known to those of ordinary skill in the art for using a binding agent to detect polypeptide markers in a sample. See, e.g., Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, 1988. In general, the presence or absence of a cancer in a patient may be determined by (a) contacting a biological sample obtained from a patient with a binding agent; (b) detecting in the sample a level of polypeptide that binds to the binding agent; and (c) comparing the level of polypeptide with a predetermined cut-off value.

In a preferred embodiment, the assay involves the use of binding agent immobilized on a solid support to bind to and remove the polypeptide from the

remainder of the sample. The bound polypeptide may then be detected using a detection reagent that contains a reporter group and specifically binds to the binding agent/polypeptide complex. Such detection reagents may comprise, for example, a binding agent that specifically binds to the polypeptide or an antibody or other agent that specifically binds to the binding agent, such as an anti-immunoglobulin, protein G, protein A or a lectin. Alternatively, a competitive assay may be utilized, in which a polypeptide is labeled with a reporter group and allowed to bind to the immobilized binding agent after incubation of the binding agent with the sample. The extent to which components of the sample inhibit the binding of the labeled polypeptide to the binding agent is indicative of the reactivity of the sample with the immobilized binding agent. Suitable polypeptides for use within such assays include full length colon tumor proteins and portions thereof to which the binding agent binds, as described above.

The solid support may be any material known to those of ordinary skill in the art to which the tumor protein may be attached. For example, the solid support may be a test well in a microtiter plate or a nitrocellulose or other suitable membrane. Alternatively, the support may be a bead or disc, such as glass, fiberglass, latex or a plastic material such as polystyrene or polyvinylchloride. The support may also be a magnetic particle or a fiber optic sensor, such as those disclosed, for example, in U.S. Patent No. 5,359,681. The binding agent may be immobilized on the solid support using a variety of techniques known to those of skill in the art, which are amply described in the patent and scientific literature. In the context of the present invention, the term "immobilization" refers to both noncovalent association, such as adsorption, and covalent attachment (which may be a direct linkage between the agent and functional groups on the support or may be a linkage by way of a cross-linking agent). Immobilization by adsorption to a well in a microtiter plate or to a membrane is preferred. In such cases, adsorption may be achieved by contacting the binding agent, in a suitable buffer, with the solid support for a suitable amount of time. The contact time varies with temperature, but is typically between about 1 hour and about 1 day. In general, contacting a well of a plastic microtiter plate (such as polystyrene or polyvinylchloride) with an amount of binding agent ranging from about 10 ng to about

10 μg , and preferably about 100 ng to about 1 μg , is sufficient to immobilize an adequate amount of binding agent.

Covalent attachment of binding agent to a solid support may generally be achieved by first reacting the support with a bifunctional reagent that will react with
5 both the support and a functional group, such as a hydroxyl or amino group, on the binding agent. For example, the binding agent may be covalently attached to supports having an appropriate polymer coating using benzoquinone or by condensation of an aldehyde group on the support with an amine and an active hydrogen on the binding partner (*see, e.g.*, Pierce Immunotechnology Catalog and Handbook, 1991, at
10 A12-A13).

In certain embodiments, the assay is a two-antibody sandwich assay. This assay may be performed by first contacting an antibody that has been immobilized on a solid support, commonly the well of a microtiter plate, with the sample, such that polypeptides within the sample are allowed to bind to the immobilized antibody.
15 Unbound sample is then removed from the immobilized polypeptide-antibody complexes and a detection reagent (preferably a second antibody capable of binding to a different site on the polypeptide) containing a reporter group is added. The amount of detection reagent that remains bound to the solid support is then determined using a method appropriate for the specific reporter group.

20 More specifically, once the antibody is immobilized on the support as described above, the remaining protein binding sites on the support are typically blocked. Any suitable blocking agent known to those of ordinary skill in the art, such as bovine serum albumin or Tween 20™ (Sigma Chemical Co., St. Louis, MO). The immobilized antibody is then incubated with the sample, and polypeptide is allowed to
25 bind to the antibody. The sample may be diluted with a suitable diluent, such as phosphate-buffered saline (PBS) prior to incubation. In general, an appropriate contact time (*i.e.*, incubation time) is a period of time that is sufficient to detect the presence of polypeptide within a sample obtained from an individual with colon cancer. Preferably, the contact time is sufficient to achieve a level of binding that is at least about 95% of
30 that achieved at equilibrium between bound and unbound polypeptide. Those of ordinary skill in the art will recognize that the time necessary to achieve equilibrium

may be readily determined by assaying the level of binding that occurs over a period of time. At room temperature, an incubation time of about 30 minutes is generally sufficient.

Unbound sample may then be removed by washing the solid support
5 with an appropriate buffer, such as PBS containing 0.1% Tween 20™. The second antibody, which contains a reporter group, may then be added to the solid support. Preferred reporter groups include those groups recited above.

The detection reagent is then incubated with the immobilized antibody-polypeptide complex for an amount of time sufficient to detect the bound polypeptide.
10 An appropriate amount of time may generally be determined by assaying the level of binding that occurs over a period of time. Unbound detection reagent is then removed and bound detection reagent is detected using the reporter group. The method employed for detecting the reporter group depends upon the nature of the reporter group. For radioactive groups, scintillation counting or autoradiographic methods are
15 generally appropriate. Spectroscopic methods may be used to detect dyes, luminescent groups and fluorescent groups. Biotin may be detected using avidin, coupled to a different reporter group (commonly a radioactive or fluorescent group or an enzyme). Enzyme reporter groups may generally be detected by the addition of substrate (generally for a specific period of time), followed by spectroscopic or other analysis of
20 the reaction products.

To determine the presence or absence of a cancer, such as colon cancer, the signal detected from the reporter group that remains bound to the solid support is generally compared to a signal that corresponds to a predetermined cut-off value. In one preferred embodiment, the cut-off value for the detection of a cancer is the average
25 mean signal obtained when the immobilized antibody is incubated with samples from patients without the cancer. In general, a sample generating a signal that is three standard deviations above the predetermined cut-off value is considered positive for the cancer. In an alternate preferred embodiment, the cut-off value is determined using a Receiver Operator Curve, according to the method of Sackett et al., *Clinical*
30 *Epidemiology: A Basic Science for Clinical Medicine*, Little Brown and Co., 1985, p. 106-7. Briefly, in this embodiment, the cut-off value may be determined from a plot

of pairs of true positive rates (*i.e.*, sensitivity) and false positive rates (100%-specificity) that correspond to each possible cut-off value for the diagnostic test result. The cut-off value on the plot that is the closest to the upper left-hand corner (*i.e.*, the value that encloses the largest area) is the most accurate cut-off value, and a sample
5 generating a signal that is higher than the cut-off value determined by this method may be considered positive. Alternatively, the cut-off value may be shifted to the left along the plot, to minimize the false positive rate, or to the right, to minimize the false negative rate. In general, a sample generating a signal that is higher than the cut-off value determined by this method is considered positive for a cancer.

10 In a related embodiment, the assay is performed in a flow-through or strip test format, wherein the binding agent is immobilized on a membrane, such as nitrocellulose. In the flow-through test, polypeptides within the sample bind to the immobilized binding agent as the sample passes through the membrane. A second, labeled binding agent then binds to the binding agent-polypeptide complex as a solution
15 containing the second binding agent flows through the membrane. The detection of bound second binding agent may then be performed as described above. In the strip test format, one end of the membrane to which binding agent is bound is immersed in a solution containing the sample. The sample migrates along the membrane through a region containing second binding agent and to the area of immobilized binding agent.
20 Concentration of second binding agent at the area of immobilized antibody indicates the presence of a cancer. Typically, the concentration of second binding agent at that site generates a pattern, such as a line, that can be read visually. The absence of such a pattern indicates a negative result. In general, the amount of binding agent immobilized on the membrane is selected to generate a visually discernible pattern when the
25 biological sample contains a level of polypeptide that would be sufficient to generate a positive signal in the two-antibody sandwich assay, in the format discussed above. Preferred binding agents for use in such assays are antibodies and antigen-binding fragments thereof. Preferably, the amount of antibody immobilized on the membrane ranges from about 25 ng to about 1 μ g, and more preferably from about 50 ng to about
30 500 ng. Such tests can typically be performed with a very small amount of biological sample.

Of course, numerous other assay protocols exist that are suitable for use with the tumor proteins or binding agents of the present invention. The above descriptions are intended to be exemplary only. For example, it will be apparent to those of ordinary skill in the art that the above protocols may be readily modified to use
5 colon tumor polypeptides to detect antibodies that bind to such polypeptides in a biological sample. The detection of such colon tumor protein specific antibodies may correlate with the presence of a cancer.

A cancer may also, or alternatively, be detected based on the presence of T cells that specifically react with a colon tumor protein in a biological sample. Within
10 certain methods, a biological sample comprising CD4⁺ and/or CD8⁺ T cells isolated from a patient is incubated with a colon tumor polypeptide, a polynucleotide encoding such a polypeptide and/or an APC that expresses at least an immunogenic portion of such a polypeptide, and the presence or absence of specific activation of the T cells is detected. Suitable biological samples include, but are not limited to, isolated T cells.
15 For example, T cells may be isolated from a patient by routine techniques (such as by Ficoll/Hypaque density gradient centrifugation of peripheral blood lymphocytes). T cells may be incubated *in vitro* for 2-9 days (typically 4 days) at 37°C with polypeptide (e.g., 5 - 25 µg/ml). It may be desirable to incubate another aliquot of a T cell sample in the absence of colon tumor polypeptide to serve as a control. For CD4⁺ T cells,
20 activation is preferably detected by evaluating proliferation of the T cells. For CD8⁺ T cells, activation is preferably detected by evaluating cytolytic activity. A level of proliferation that is at least two fold greater and/or a level of cytolytic activity that is at least 20% greater than in disease-free patients indicates the presence of a cancer in the patient.

25 As noted above, a cancer may also, or alternatively, be detected based on the level of mRNA encoding a colon tumor protein in a biological sample. For example, at least two oligonucleotide primers may be employed in a polymerase chain reaction (PCR) based assay to amplify a portion of a colon tumor cDNA derived from a biological sample, wherein at least one of the oligonucleotide primers is specific for
30 (i.e., hybridizes to) a polynucleotide encoding the colon tumor protein. The amplified cDNA is then separated and detected using techniques well known in the art, such as

gel electrophoresis. Similarly, oligonucleotide probes that specifically hybridize to a polynucleotide encoding a colon tumor protein may be used in a hybridization assay to detect the presence of polynucleotide encoding the tumor protein in a biological sample.

To permit hybridization under assay conditions, oligonucleotide primers
5 and probes should comprise an oligonucleotide sequence that has at least about 60%, preferably at least about 75% and more preferably at least about 90%, identity to a portion of a polynucleotide encoding a colon tumor protein that is at least 10 nucleotides, and preferably at least 20 nucleotides, in length. Preferably, oligonucleotide primers and/or probes hybridize to a polynucleotide encoding a
10 polypeptide described herein under moderately stringent conditions, as defined above. Oligonucleotide primers and/or probes which may be usefully employed in the diagnostic methods described herein preferably are at least 10-40 nucleotides in length. In a preferred embodiment, the oligonucleotide primers comprise at least 10 contiguous nucleotides, more preferably at least 15 contiguous nucleotides, of a DNA molecule
15 having a sequence recited in SEQ ID NOs:1-1556. Techniques for both PCR based assays and hybridization assays are well known in the art (*see*, for example, Mullis et al., *Cold Spring Harbor Symp. Quant. Biol.*, 51:263, 1987; Erlich ed., *PCR Technology*, Stockton Press, NY, 1989).

One preferred assay employs RT-PCR, in which PCR is applied in
20 conjunction with reverse transcription. Typically, RNA is extracted from a biological sample, such as biopsy tissue, and is reverse transcribed to produce cDNA molecules. PCR amplification using at least one specific primer generates a cDNA molecule, which may be separated and visualized using, for example, gel electrophoresis. Amplification may be performed on biological samples taken from a test patient and
25 from an individual who is not afflicted with a cancer. The amplification reaction may be performed on several dilutions of cDNA spanning two orders of magnitude. A two-fold or greater increase in expression in several dilutions of the test patient sample as compared to the same dilutions of the non-cancerous sample is typically considered positive.

30 In another embodiment, the compositions described herein may be used as markers for the progression of cancer. In this embodiment, assays as described

above for the diagnosis of a cancer may be performed over time, and the change in the level of reactive polypeptide(s) or polynucleotide(s) evaluated. For example, the assays may be performed every 24-72 hours for a period of 6 months to 1 year, and thereafter performed as needed. In general, a cancer is progressing in those patients in whom the level of polypeptide or polynucleotide detected increases over time. In contrast, the cancer is not progressing when the level of reactive polypeptide or polynucleotide either remains constant or decreases with time.

Certain *in vivo* diagnostic assays may be performed directly on a tumor. One such assay involves contacting tumor cells with a binding agent. The bound binding agent may then be detected directly or indirectly via a reporter group. Such binding agents may also be used in histological applications. Alternatively, polynucleotide probes may be used within such applications.

As noted above, to improve sensitivity, multiple colon tumor protein markers may be assayed within a given sample. It will be apparent that binding agents specific for different proteins provided herein may be combined within a single assay. Further, multiple primers or probes may be used concurrently. The selection of tumor protein markers may be based on routine experiments to determine combinations that results in optimal sensitivity. In addition, or alternatively, assays for tumor proteins provided herein may be combined with assays for other known tumor antigens.

20 DIAGNOSTIC KITS

The present invention further provides kits for use within any of the above diagnostic methods. Such kits typically comprise two or more components necessary for performing a diagnostic assay. Components may be compounds, reagents, containers and/or equipment. For example, one container within a kit may contain a monoclonal antibody or fragment thereof that specifically binds to a colon tumor protein. Such antibodies or fragments may be provided attached to a support material, as described above. One or more additional containers may enclose elements, such as reagents or buffers, to be used in the assay. Such kits may also, or alternatively, contain a detection reagent as described above that contains a reporter group suitable for direct or indirect detection of antibody binding.

Alternatively, a kit may be designed to detect the level of mRNA encoding a colon tumor protein in a biological sample. Such kits generally comprise at least one oligonucleotide probe or primer, as described above, that hybridizes to a polynucleotide encoding a colon tumor protein. Such an oligonucleotide may be used, for example, within a PCR or hybridization assay. Additional components that may be present within such kits include a second oligonucleotide and/or a diagnostic reagent or container to facilitate the detection of a polynucleotide encoding a colon tumor protein.

The following Examples are offered by way of illustration and not by way of limitation.

EXAMPLE 1

IDENTIFICATION OF COLON TUMOR PROTEIN CDNAS

This Example illustrates the identification of cDNA molecules encoding
5 colon tumor proteins using PCR-based cDNA subtraction methodology.

A pool of tester mRNA was collected from three colon adenocarcinoma
samples showing moderate histological differentiation and no evidence of metastasis.
Eight normal tissues, including brain, pancreas, bone marrow, liver, heart, lung,
stomach and small intestine were represented in the driver mRNA pool. cDNA
10 synthesis, hybridization and PCR amplification were performed according to the
methods of Clontech (Palo Alto, CA), with minor modifications. In a first subtraction,
the restriction enzymes PvuII, DraI, MscI and StuI were used to digest cDNAs. The
tester to driver ratio was 1:40. In a second subtraction, DraI, MscI and StuI were used
for cDNA digestion. A tester to driver ratio of 1:76 was employed. Following the PCR
15 amplification steps, the cDNAs were cloned into the pCR2.1 plasmid vector. The
libraries resulting from the first and second subtractions, named CPS1 and CPS2,
respectively, were used to obtain clones for microarray analysis and sequencing. Inserts
were PCR amplified and purified. Each clone was sequenced from one direction with
either M13 Forward primer or M13 Reverse primer. The determined cDNA sequences
20 for 1535 of the isolated clones are provided in SEQ ID NOs:1-1556.

A cDNA library was constructed in the PCR2.1 vector (Invitrogen,
Carlsbad, CA) by subtracting a pool of three colon tumors with a pool of normal colon,
spleen, brain, liver, kidney, lung, stomach and small intestine using PCR subtraction
methodologies (Clontech, Palo Alto, CA). The subtraction was performed using a
25 PCR-based protocol, which was modified to generate larger fragments. Within this
protocol, tester and driver double stranded cDNA were separately digested with five
restriction enzymes that recognize six-nucleotide restriction sites (MluI, MscI, PvuII,
SalI and StuI). This digestion resulted in an average cDNA size of 600 bp, rather than
the average size of 300 bp that results from digestion with RsaI according to the
30 Clontech protocol. This modification did not affect the subtraction efficiency. Two

tester populations were then created with different adapters, and the driver library remained without adapters.

The tester and driver libraries were then hybridized using excess driver cDNA. In the first hybridization step, driver was separately hybridized with each of the two tester cDNA populations. This resulted in populations of (a) unhybridized tester cDNAs, (b) tester cDNAs hybridized to other tester cDNAs, (c) tester cDNAs hybridized to driver cDNAs, and (d) unhybridized driver cDNAs. The two separate hybridization reactions were then combined, and rehybridized in the presence of additional denatured driver cDNA. Following this second hybridization, in addition to populations (a) through (d), a fifth population (e) was generated in which tester cDNA with one adapter hybridized to tester cDNA with the second adapter. Accordingly, the second hybridization step resulted in enrichment of differentially expressed sequences which could be used as templates for PCR amplification with adaptor-specific primers.

The ends were then filled in, and PCR amplification was performed using adaptor-specific primers. Only population (e), which contained tester cDNA that did not hybridize to driver cDNA, was amplified exponentially. A second PCR amplification step was then performed, to reduce background and further enrich differentially expressed sequences.

This PCR-based subtraction technique normalizes differentially expressed cDNAs so that rare transcripts that are over-expressed in colon tumor tissue may be recoverable. Such transcripts would be difficult to recover by traditional subtraction methods.

To characterize the complexity and redundancy of the subtracted library, 96 clones were randomly picked and 65 were sequenced, as previously described. These sequences were further characterized by comparison with the most recent Genbank database (April, 1998) to determine their degree of novelty. No significant homologies were found to 21 of these clones, hereinafter referred to as 11092, 11093, 11096, 11098, 11103, 11174, 11108, 11112, 11115, 11117, 11118, 11134, 11151, 11154, 11158, 11168, 11172, 11175, 11184, 11185 and 11187. The determined cDNA sequences for these clones are provided in SEQ ID NO: 48, 49, 52, 54, 59, 60, 65-69, 79, 89, 90, 93, 99-101 and 109-111, respectively.

Two-thousand clones from the above mentioned cDNA subtraction library were randomly picked and submitted to a round of PCR amplification. Briefly, 0.5 µl of glycerol stock solution was added to 99.5 µl of pcr MIX (80 µl H₂O, 10 µl 10X PCR Buffer, 6 µl 25 mM MgCl₂, 1 µl 10 mM dNTPs, 1 µl 100 mM M13 forward primer (CACGACGTTGTAAAACGACGG), 1 µl 100 mM M13 reverse primer (CACAGGAAACAGCTATGACC)), and 0.5 µl 5 u/ml Taq polymerase (primers provided by (Operon Technologies, Alameda, CA). The PCR amplification was run for thirty cycles under the following conditions: 95°C for 5 min., 92°C for 30 sec., 57°C for 40 sec., 75°C for 2 min. and 75°C for 5 minutes.

mRNA expression levels for representative clones were determined using microarray technology (Synteni, Palo Alto, CA) in colon tumor tissues (n=25), normal colon tissues (n=6), kidney, lung, liver, brain, heart, esophagus, small intestine, stomach, pancreas, adrenal gland, salivary gland, resting PBMC, activated PBMC, bone marrow, dendritic cells, spinal cord, blood vessels, skeletal muscle, skin, breast and fetal tissues. The number of tissue samples tested in each case was one (n=1), except where specifically noted above; additionally, all the above-mentioned tissues were derived from humans. The PCR amplification products were dotted onto slides in an array format, with each product occupying a unique location in the array. mRNA was extracted from the tissue sample to be tested, and fluorescent-labeled cDNA probes were generated by reverse transcription according to the protocol provided by Synteni. The microarrays were probed with the labeled cDNA probes, the slides scanned, and fluorescence intensity was measured. This intensity correlates with the hybridization intensity.

Clones corresponding to SEQ ID Nos:1506-1556 were overexpressed in colon tumors and showed low or no expression levels in normal tissues.

EXAMPLE 2

SYNTHESIS OF POLYPEPTIDES

Polypeptides may be synthesized on a Perkin Elmer/Applied Biosystems Division 430A peptide synthesizer using Fmoc chemistry with HPTU (O-

Benzotriazole-N,N,N',N'-tetramethyluronium hexafluorophosphate) activation. A Gly-Cys-Gly sequence may be attached to the amino terminus of the peptide to provide a method of conjugation, binding to an immobilized surface, or labeling of the peptide. Cleavage of the peptides from the solid support may be carried out using the following

5 cleavage mixture: trifluoroacetic acid:ethanedithiol:thioanisole:water:phenol (40:1:2:2:3). After cleaving for 2 hours, the peptides may be precipitated in cold methyl-t-butyl-ether. The peptide pellets may then be dissolved in water containing 0.1% trifluoroacetic acid (TFA) and lyophilized prior to purification by C18 reverse phase HPLC. A gradient of 0%-60% acetonitrile (containing 0.1% TFA) in water

10 (containing 0.1% TFA) may be used to elute the peptides. Following lyophilization of the pure fractions, the peptides may be characterized using electrospray or other types of mass spectrometry and by amino acid analysis.

From the foregoing it will be appreciated that, although specific embodiments of the invention have been described herein for purposes of illustration,

15 various modifications may be made without deviating from the spirit and scope of the invention. Accordingly, the invention is not limited except as by the appended claims.

CLAIMS

1. An isolated polypeptide, comprising at least an immunogenic portion of a colon tumor protein, or a variant thereof, wherein the tumor protein comprises an amino acid sequence that is encoded by a polynucleotide sequence selected from the group consisting of:

- (a) sequences recited in SEQ ID NOs:1-1556;
- (b) sequences that hybridize to a sequence recited in any one of SEQ ID NOs:1-1556 under moderately stringent conditions; and
- (c) complements of sequences of (a) or (b).

2. An isolated polypeptide according to claim 1, wherein the polypeptide comprises an amino acid sequence that is encoded by a polynucleotide sequence recited in any one of SEQ ID NOs:1-1556 or a complement of any of the foregoing polynucleotide sequences.

3. An isolated polynucleotide encoding at least 15 amino acid residues of a colon tumor protein, or a variant thereof that differs in one or more substitutions, deletions, additions and/or insertions such that the ability of the variant to react with antigen-specific antisera is not substantially diminished, wherein the tumor protein comprises an amino acid sequence that is encoded by a polynucleotide comprising a sequence recited in any one of SEQ ID NOs:1-1556 or a complement of any of the foregoing sequences.

4. An isolated polynucleotide encoding a colon tumor protein, or a variant thereof, wherein the tumor protein comprises an amino acid sequence that is encoded by a polynucleotide comprising a sequence recited in any one of SEQ ID NOs:1-1556 or a complement of any of the foregoing sequences.

5. An isolated polynucleotide, comprising a sequence recited in any one of SEQ ID NOs:1-1556.
6. An isolated polynucleotide, comprising a sequence that hybridizes to a sequence recited in any one of SEQ ID NOs:1-1556 under moderately stringent conditions.
7. An isolated polynucleotide complementary to a polynucleotide according to any one of claims 3-6.
8. An expression vector, comprising a polynucleotide according to any one of claims 3-7.
9. A host cell transformed or transfected with an expression vector according to claim 8.
10. An isolated antibody, or antigen-binding fragment thereof, that specifically binds to a colon tumor protein that comprises an amino acid sequence that is encoded by a polynucleotide sequence recited in any one of SEQ ID NOs1-1556 or a complement of any of the foregoing polynucleotide sequences.
11. A fusion protein, comprising at least one polypeptide according to claim 1.
12. A fusion protein according to claim 11, wherein the fusion protein comprises an expression enhancer that increases expression of the fusion protein in a host cell transfected with a polynucleotide encoding the fusion protein.

13. A fusion protein according to claim 11, wherein the fusion protein comprises a T helper epitope that is not present within the polypeptide of claim 1.

14. A fusion protein according to claim 11, wherein the fusion protein comprises an affinity tag.

15. An isolated polynucleotide encoding a fusion protein according to claim 11.

16. A pharmaceutical composition, comprising a physiologically acceptable carrier and at least one component selected from the group consisting of:

- (a) a polypeptide according to claim 1;
- (b) a polynucleotide according to claim 3;
- (c) an antibody according to claim 10;
- (d) a fusion protein according to claim 11; and
- (e) a polynucleotide according to claim 15.

17. A vaccine comprising an immunostimulant and at least one component selected from the group consisting of:

- (a) a polypeptide according to claim 1;
- (b) a polynucleotide according to claim 3;
- (c) an antibody according to claim 10;
- (d) a fusion protein according to claim 11; and
- (e) a polynucleotide according to claim 15.

18. A vaccine according to claim 17, wherein the immunostimulant is an adjuvant.

19. A vaccine according to any claim 17, wherein the immunostimulant induces a predominantly Type I response.

20. A method for inhibiting the development of a cancer in a patient, comprising administering to a patient an effective amount of a pharmaceutical composition according to claim 16.

21. A method for inhibiting the development of a cancer in a patient, comprising administering to a patient an effective amount of a vaccine according to claim 17.

22. A pharmaceutical composition comprising an antigen-presenting cell that expresses a polypeptide according to claim 1, in combination with a pharmaceutically acceptable carrier or excipient.

23. A pharmaceutical composition according to claim 22, wherein the antigen presenting cell is a dendritic cell or a macrophage.

24. A vaccine comprising an antigen-presenting cell that expresses a polypeptide comprising at least an immunogenic portion of a colon tumor protein, or a variant thereof, wherein the tumor protein comprises an amino acid sequence that is encoded by a polynucleotide sequence selected from the group consisting of:

- (a) sequences recited in SEQ ID NOs:1-1556;
 - (b) sequences that hybridize to a sequence recited in any one of SEQ ID NOs:1-1556 under moderately stringent conditions; and
 - (c) complements of sequences of (i) or (ii);
- in combination with an immunostimulant.

25. A vaccine according to claim 24, wherein the immunostimulant is an adjuvant.

26. A vaccine according to claim 24, wherein the immunostimulant induces a predominantly Type I response.

27. A vaccine according to claim 24, wherein the antigen-presenting cell is a dendritic cell.

28. A method for inhibiting the development of a cancer in a patient, comprising administering to a patient an effective amount of an antigen-presenting cell that expresses a polypeptide comprising at least an immunogenic portion of a colon tumor protein, or a variant thereof, wherein the tumor protein comprises an amino acid sequence that is encoded by a polynucleotide sequence selected from the group consisting of:

- (a) sequences recited in SEQ ID NOs:1-1556;
 - (b) sequences that hybridize to a sequence recited in any one of SEQ ID NOs:1-1556 under moderately stringent conditions; and
 - (c) complements of sequences encoded by a polynucleotide recited in any one of SEQ ID NOs:1-1556;
- and thereby inhibiting the development of a cancer in the patient.

29. A method according to claim 28, wherein the antigen-presenting cell is a dendritic cell.

30. A method according to any one of claims 20, 21 and 28, wherein the cancer is colon cancer.

31. A method for removing tumor cells from a biological sample, comprising contacting a biological sample with T cells that specifically react with a colon tumor protein, wherein the tumor protein comprises an amino acid sequence that is encoded by a polynucleotide sequence selected from the group consisting of:

- (i) polynucleotides recited in any one of SEQ ID NOs:1-1556; and

(ii) complements of the foregoing polynucleotides;

wherein the step of contacting is performed under conditions and for a time sufficient to permit the removal of cells expressing the antigen from the sample.

32. A method according to claim 31, wherein the biological sample is blood or a fraction thereof.

33. A method for inhibiting the development of a cancer in a patient, comprising administering to a patient a biological sample treated according to the method of claim 32.

34. A method for stimulating and/or expanding T cells specific for a colon tumor protein, comprising contacting T cells with at least one component selected from the group consisting of:

(a) polypeptides comprising at least an immunogenic portion of a colon tumor protein, or a variant thereof, wherein the tumor protein comprises an amino acid sequence that is encoded by a polynucleotide sequence selected from the group consisting of:

(i) sequences recited in SEQ ID NOs:1-1556;

(ii) sequences that hybridize to a sequence recited in any one of SEQ ID NOs:1-1556 under moderately stringent conditions; and

(iii) complements of sequences of (i) or (ii);

(b) polynucleotides encoding a polypeptide of (a); and

(c) antigen presenting cells that express a polypeptide of (a);

under conditions and for a time sufficient to permit the stimulation and/or expansion of T cells.

35. An isolated T cell population, comprising T cells prepared according to the method of claim 34.

36. A method for inhibiting the development of a cancer in a patient, comprising administering to a patient an effective amount of a T cell population according to claim 35.

37. A method for inhibiting the development of a cancer in a patient, comprising the steps of:

(a) incubating CD4⁺ and/or CD8⁺ T cells isolated from a patient with at least one component selected from the group consisting of:

(i) polypeptides comprising at least an immunogenic portion of a colon tumor protein, or a variant thereof, wherein the tumor protein comprises an amino acid sequence that is encoded by a polynucleotide sequence selected from the group consisting of:

(1) sequences recited in SEQ ID NOs:1-1556;

(2) sequences that hybridize to a sequence recited in any one of SEQ ID NOs:1-1556 under moderately stringent conditions; and

(3) complements of sequences of (1) or (2);

(ii) polynucleotides encoding a polypeptide of (i); and

(iii) antigen presenting cells that expresses a polypeptide of (i);

such that T cells proliferate; and

(b) administering to the patient an effective amount of the proliferated T cells, and thereby inhibiting the development of a cancer in the patient.

38. A method for inhibiting the development of a cancer in a patient, comprising the steps of:

(a) incubating CD4⁺ and/or CD8⁺ T cells isolated from a patient with at least one component selected from the group consisting of:

(i) polypeptides comprising at least an immunogenic portion of a colon tumor protein, or a variant thereof, wherein the tumor protein comprises an

amino acid sequence that is encoded by a polynucleotide sequence selected from the group consisting of:

- (1) sequences recited in SEQ ID NOs:1-1556;
 - (2) sequences that hybridize to a sequence recited in any one of SEQ ID NOs:1-1556 under moderately stringent conditions; and
 - (3) complements of sequences of (1) or (2);
 - (ii) polynucleotides encoding a polypeptide of (i); and
 - (iii) antigen presenting cells that express a polypeptide of (i);
- such that T cells proliferate;

(b) cloning at least one proliferated cell to provide cloned T cells;

and

(c) administering to the patient an effective amount of the cloned T cells, and thereby inhibiting the development of a cancer in the patient.

39. A method for determining the presence or absence of a cancer in a patient, comprising the steps of:

(a) contacting a biological sample obtained from a patient with a binding agent that binds to a colon tumor protein, wherein the tumor protein comprises an amino acid sequence that is encoded by a polynucleotide sequence recited in any one of SEQ ID NOs:1-1556 or a complement of any of the foregoing polynucleotide sequences;

(b) detecting in the sample an amount of polypeptide that binds to the binding agent; and

(c) comparing the amount of polypeptide to a predetermined cut-off value, and therefrom determining the presence or absence of a cancer in the patient.

40. A method according to claim 39, wherein the binding agent is an antibody.

41. A method according to claim 42, wherein the antibody is a monoclonal antibody.
42. A method according to claim 39, wherein the cancer is colon cancer.
43. A method for monitoring the progression of a cancer in a patient, comprising the steps of:
- (a) contacting a biological sample obtained from a patient at a first point in time with a binding agent that binds to a colon tumor protein, wherein the tumor protein comprises an amino acid sequence that is encoded by a polynucleotide sequence recited in any one of SEQ ID NOs:1-1556 or a complement of any of the foregoing polynucleotide sequences;
 - (b) detecting in the sample an amount of polypeptide that binds to the binding agent;
 - (c) repeating steps (a) and (b) using a biological sample obtained from the patient at a subsequent point in time; and
 - (d) comparing the amount of polypeptide detected in step (c) to the amount detected in step (b) and therefrom monitoring the progression of the cancer in the patient.
44. A method according to claim 43, wherein the binding agent is an antibody.
45. A method according to claim 44, wherein the antibody is a monoclonal antibody.
46. A method according to claim 43, wherein the cancer is a colon cancer.

47. A method for determining the presence or absence of a cancer in a patient, comprising the steps of:

(a) contacting a biological sample obtained from a patient with an oligonucleotide that hybridizes to a polynucleotide that encodes a colon tumor protein, wherein the tumor protein comprises an amino acid sequence that is encoded by a polynucleotide sequence recited in any one of SEQ ID NO:1-1556 or a complement of any of the foregoing polynucleotide sequences;

(b) detecting in the sample an amount of a polynucleotide that hybridizes to the oligonucleotide; and

(c) comparing the amount of polynucleotide that hybridizes to the oligonucleotide to a predetermined cut-off value, and therefrom determining the presence or absence of a cancer in the patient.

48. A method according to claim 47, wherein the amount of polynucleotide that hybridizes to the oligonucleotide is determined using a polymerase chain reaction.

49. A method according to claim 47, wherein the amount of polynucleotide that hybridizes to the oligonucleotide is determined using a hybridization assay.

50. A method for monitoring the progression of a cancer in a patient, comprising the steps of:

(a) contacting a biological sample obtained from a patient with an oligonucleotide that hybridizes to a polynucleotide that encodes a colon tumor protein, wherein the tumor protein comprises an amino acid sequence that is encoded by a polynucleotide sequence recited in any one of SEQ ID NO:1-1556 or a complement of any of the foregoing polynucleotide sequences;

(b) detecting in the sample an amount of a polynucleotide that hybridizes to the oligonucleotide;

(c) repeating steps (a) and (b) using a biological sample obtained from the patient at a subsequent point in time; and

(d) comparing the amount of polynucleotide detected in step (c) to the amount detected in step (b) and therefrom monitoring the progression of the cancer in the patient.

51. A method according to claim 50, wherein the amount of polynucleotide that hybridizes to the oligonucleotide is determined using a polymerase chain reaction.

52. A method according to claim 50, wherein the amount of polynucleotide that hybridizes to the oligonucleotide is determined using a hybridization assay.

53. A diagnostic kit, comprising:

- (a) one or more antibodies according to claim 10; and
- (b) a detection reagent comprising a reporter group.

54. A kit according to claim 53, wherein the antibodies are immobilized on a solid support.

55. A kit according to claim 53, wherein the detection reagent comprises an anti-immunoglobulin, protein G, protein A or lectin.

56. A kit according to claim 53, wherein the reporter group is selected from the group consisting of radioisotopes, fluorescent groups, luminescent groups, enzymes, biotin and dye particles.

57. An oligonucleotide comprising 10 to 40 contiguous nucleotides that hybridize under moderately stringent conditions to a polynucleotide that encodes a

colon tumor protein, wherein the tumor protein comprises an amino acid sequence that is encoded by a polynucleotide sequence recited in any one of SEQ ID NOs:1-1556 or a complement of any of the foregoing polynucleotides.

58. A oligonucleotide according to claim 57, wherein the oligonucleotide comprises 10-40 contiguous nucleotides recited in any one of SEQ ID NOs:1-1556.

59. A diagnostic kit, comprising:

- (a) an oligonucleotide according to claim 58; and
- (b) a diagnostic reagent for use in a polymerase chain reaction or hybridization assay.

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300agctntgagt ggctcttggg ccaggcttgt tccatctnt ccttctctt nccaactccc
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451

<210> 16<211> 452<212> DNA<213> Homo sapien
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120aagttctggt tttgaacttt gtggaactgt tccaatcaat caatttccca gttatgatga
180gtattttacat tatgaatgta taaccagac atgatttgta aagccgacag tatgtttcta
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420atnggagngg ggggaaaact gnnttttaan cn
452

<210> 17<211> 244<212> DNA<213> Homo sapien
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120agaacctcta caggttatgc agcaagttct taatattatt tcccttctgt ggaaactctc
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240tttt
244

<210> 18<211> 84<212> DNA<213> Homo sapien
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84

<210> 19<211> 312<212> DNA<213> Homo sapien
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120agttccggg tcttgggcc ctcttcaagt cctgcctga gcccggtggc ctcaccgagt
180cagagacgga gtatgtcatc cgctgcacca aacacacctt caccaaccac atggtttttc
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300agcccactga gg
312

<210> 20<211> 420<212> DNA<213> Homo sapien
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120tcccatcggc ttgatctctt gtatctgcc aacgcttttn ctctgcaaaa agcctgggat
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240caacgtctgc cttcgctagg ctgacatcgc gggacccgc tgccttcctc tcttcaactg
300ccacatagac ggntgcaatc tctccatana agtggncctg cttcacttca caccagtacc
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420

<210> 21<211> 435<212> DNA<213> Homo sapien .

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120gcccaccaag ctgtcactgc tgcactcact ctgcaaggga tcaggaccag caacctttat
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240caaataccaa gaatttttgc gtatgtttat attgtatngt tctaaataat gggtagnctg
300tgaaataaga tcttgccacc catgtaataa tantagtaat actatagtna naaatggctg
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435

<210> 22<211> 407<212> DNA<213> Homo sapien

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120gccacccac ttacatttcc tactatacaa tgcctttttg gcgcttgata aatcaagcat
180tcatgtagca ttacattcaa cagaaacatt tctcgtactt tgggtttaag atccttgtcc
240ctccagntcg gatgtcgtga catctgactc ttcntcattg taaatatttt canccatttg
300ccatatctgc atgatgttat cctcagacac tgagcaaatg acccaaggct nattgggggt
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407

<210> 23<211> 272<212> DNA<213> Homo sapien

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120caaaaacctg tttttgaatc cccaagaagg cagcatgtgt atacaacct accacatttg
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272

<210> 24<211> 424<212> DNA<213> Homo sapien

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120agataagaaa gttgaaacaa aggtttgtta agtctcctga actattcagt ttaataacca
180attatccctc aatattacaa aataaaatga ctggatcaat gntgactctt ctttgatctc
240atttataaaa tgctttgata ttacttacta agttccctga taactcaaac aaggtaaaat
300taacactttc attttcagggt attgtctatt tagaagagga ttaccacttt catttagatg
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424

<210> 25<211> 372<212> DNA<213> Homo sapien

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240cctcattgat ggnccctggct gatggnggt gagcatctgn tcccatccca caccgatng
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372

<210> 26<211> 342<212> DNA<213> Homo sapien

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120atctgggaaa tgaatgaata agtactctgt ggctaccagt tgcattatgg agtcacctag
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240gaatgccctt gccagaagtn gaacatgagt aaaaattact ccaattgctt cttctaactc
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342

<210> 27<211> 315<212> DNA<213> Homo sapien

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120gggtttggtt anacgtccgg gaattgcac tggttttaag cctaattgtg ggacagctca
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240tactcgattg tcaacgtcaa ggagtcgag gtcgcctggt tctaggaata atgggggaag
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315

<210> 28<211> 311<212> DNA<213> Homo sapien

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240tgnatnactat aaaaaggaca tntgtgctcg cttgtnagaa aactctcngn tttcacgngn
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311

<210> 29<211> 516<212> DNA<213> Homo sapien

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120cacagagttt gtaatagttg ttgtggacag tacagacaga gagaggattt ctgtaactag
180agaagaactc tataaatgt tagcgcata ggaactaaaa aaagctggat tgctgatttt
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420gacctcttct ctgatttttg tataaatgaa ggtgctggac tttacctgaa agctgcaaaa
480attaatggtt tagatatatt nataataaac tgattt
516

<210> 30<211> 355<212> DNA<213> Homo sapien

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120gcctgttccc tctgcgccag aagaaggccc acctgatgga gatccagggt aacggaggca
180ctgtggccga gaagctggac tgggcccgcg agaggcttga gcagcaggta cctgtgaacc
240aagtgtttgg gcaggatgag atgatcgacg tcatcggggg gaccaagggc aaaggctaca
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355

<210> 31<211> 355<212> DNA<213> Homo sapien

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120ttacttcatg tgggtctgga tggaagatta gtggccctac aggatcattt atttatattg
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355

<210> 32<211> 285<212> DNA<213> Homo sapien

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120gcagggcacg ctctgtggtc ctccgtgcca ccggatcag gtagaccatg gaggctccca
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240gcaggggagg attctcctgc tggcgggaagc agcggagcat gcagg
285

<210> 33<211> 250<212> DNA<213> Homo sapien

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180gacggagccc tggcatgtca acagcgttcc tagagaagac aggctggaag atagctgtga
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250

<210> 34<211> 455<212> DNA<213> Homo sapien
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300ccgccctctt ggtgaggaca atgtctgcta tntcaacac cacatgagca tatnnttctg
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455

<210> 35<211> 409<212> DNA<213> Homo sapien
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300gaaacttacc caagcaacgt aattcctgtt ttcattgggtc ctgtanagt ttgagtcacg
360aagggaangc nggggagtga ctgaataaac tctgntttt acctcggca
409

<210> 36<211> 225<212> DNA<213> Homo sapien
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120tttcaacttc cagctttctg tctaagcct gtgtgccaat ggcttgagtt aggtctgctc
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225

<210> 37<211> 267<212> DNA<213> Homo sapien
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240gtttngcggg tccttcaca gnagcgg
267

<210> 38<211> 556<212> DNA<213> Homo sapien
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180aaaattaact ttagaataag ggaaggatgt tgtgtaaaat caagtcaatt atttgagnt
240tttataatat tgagtactta tgtactaagt cacaccagc cagtcaataa ctgagaaac
300aaaataaaat aataattca aagaattaca taaatacagg gccttttgag atttttgna
360ttgttaaaaca aaacgaatgg tttttacaat tcagtgtaat tctacgaata tttatttggc
420ccatgttag gcaactganc tncacagcaa gngaaataga cctcggnncg cgaccacgt
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556

<210> 39<211> 203<212> DNA<213> Homo sapien
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203

<210> 40<211> 560<212> DNA<213> Homo sapien
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560

<210> 41<211> 265<212> DNA<213> Homo sapien
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120ggacctccat ggaaggcagt ttgtgaata tccggagaac ttggaagtga ctttcaggat
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265

<210> 42<211> 407<212> DNA<213> Homo sapien
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407

<210> 43<211> 343<212> DNA<213> Homo sapien
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343

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186

<210> 45<211> 503<212> DNA<213> Homo sapien
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503

<210> 46<211> 559<212> DNA<213> Homo sapien
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<210> 47<211> 513<212> DNA<213> Homo sapien
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 513

<210> 48<211> 413<212> DNA<213> Homo sapien
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 560

<210> 50<211> 231<212> DNA<213> Homo sapien
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 120agccaagggt ggtgtccatt tctgggaatg gttaaacaca aaaggctgat agctggtatc
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<210> 51<211> 265<212> DNA<213> Homo sapien
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120tcctctgatac aggggtgagca tcaaaactcaa actacgccct gatcgggcgca ctgcgagcag
180tagcccaaac aatctcatat gaagtcaccc tagccatcat tctactatca acattactaa
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318

<210> 53<211> 335<212> DNA<213> Homo sapien
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120cgccagttcc ccaagtggtt ggggggcttt gaccgagtag tgcctggatgc tccctgcagt
180ggcactggg tcatctccaa ggatccagcc gtgaagacta acaaggatga gaaggacatc
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<210> 54<211> 280<212> DNA<213> Homo sapien
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120gaagcctctt acaaaattat ttacatcggt tgcattctat ttacatcttt taagagcaac
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280

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420tacctcttga tgtcacctt ccttgccnat gccaggacc acgaaanaaa tanccttgc
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<210> 56<211> 448<212> DNA<213> Homo sapien
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448

<210> 57<211> 454<212> DNA<213> Homo sapien
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454

<210> 58<211> 364<212> DNA<213> Homo sapien
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120ccccactccc ctgtaacttg cctgtctcat catcgctccc agtcacctca taatgacctt
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240ctttcccacc ttccagcgac acttcccac cttcaaaactc cagcgtcccc gtcgggggtg
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364

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368

<210> 60<211> 440<212> DNA<213> Homo sapien
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300cactaataca tacacgtttt tcagtgcagn aacattgaaa taatccttgt atnctttatt
360cagacagntc tgcaactag ggaataaatn aagatacgaa cacacaagaa ctttttttta
420cctcggcccg cgaccacgc
440

240gaagggcatg ctctcttctc tctctctgct gcccacttc ccgcoctgca acgtgttgaa
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<210> 65<211> 433<212> DNA<213> Homo sapien
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<210> 66<211> 517<212> DNA<213> Homo sapien
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 120gggagtacac aaatgaattg aatattggat cagaatttac cctaacttga agagttaaaa
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 240aaaaattgga atatattatg agcactgcca attgctcatt ttgtctgata ttaacagatt
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<210> 67<211> 558<212> DNA<213> Homo sapien
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 120cagcaccatt cacaagcag gcaatgttcc catctagtcc tatgtatttt agatcatatt
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<210> 68<211> 347<212> DNA<213> Homo sapien
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<210> 70<211> 530<212> DNA<213> Homo sapien
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420gagatacttt aagtcattt cttttcaaag ggggtntaat gctcanagt ggnaatagn
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530

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484

<210> 72<211> 325<212> DNA<213> Homo sapien
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325

<210> 73<211> 255<212> DNA<213> Homo sapien
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255

<210> 74<211> 244<212> DNA<213> Homo sapien
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244

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301

<210> 77<211> 335<212> DNA<213> Homo sapien
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<210> 81<211> 570<212> DNA<213> Homo sapien
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570

<210> 82<211> 567<212> DNA<213> Homo sapien
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<210> 84<211> 234<212> DNA<213> Homo sapien
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<210> 85<211> 306<212> DNA<213> Homo sapien
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306

<210> 86<211> 318<212> DNA<213> Homo sapien
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318

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180aaatgacttc tgaggaaagg gaattgtatg catcaatgat caactgaatt acattgctag
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435

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120tatactatgg gaaaccaaga aggaagggga gaaaaaaaag aaaaagcaca gtctatccat
180ccaggacaat cagtaaaaat ctacagtaac ctgatcaacc aaaaatcctt aggtgtgtg
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293

<210> 89<211> 264<212> DNA<213> Homo sapien
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120acctgaactc aggaagaaaa tgcttatctt gatgaaata tcaacagccc acccagta
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321

<210> 91<211> 208<212> DNA<213> Homo sapien
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208

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120caacaacaag ggcaagtaat agcagcttcc tttttgtcac tctccattc ccaagtggtg
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201

<210> 93<211> 387<212> DNA<213> Homo sapien
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240gaactgagga gatttgcgaa tacactgtcg caattcatca taaacatttt ctttgtcaaa
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387

<210> 94<211> 233<212> DNA<213> Homo sapien
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233

<210> 95<211> 268<212> DNA<213> Homo sapien
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268

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178

<210> 97<211> 338<212> DNA<213> Homo sapien
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338

<210> 98<211> 373<212> DNA<213> Homo sapien
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373

<210> 99<211> 344<212> DNA<213> Homo sapien
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344

<210> 100<211> 264<212> DNA<213> Homo sapien
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264

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409

<210> 102<211> 185<212> DNA<213> Homo sapien
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185

<210> 103<211> 477<212> DNA<213> Homo sapien
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477

<210> 104<211> 575<212> DNA<213> Homo sapien

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575

<210> 105<211> 286<212> DNA<213> Homo sapien

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286

<210> 106<211> 410<212> DNA<213> Homo sapien

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410

<210> 107<211> 146<212> DNA<213> Homo sapien

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146

<210> 108<211> 273<212> DNA<213> Homo sapien

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273

<210> 109<211> 264<212> DNA<213> Homo sapien

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264

<210> 110<211> 410<212> DNA<213> Homo sapien

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410

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577

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154

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396

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344

<210> 115<211> 542<212> DNA<213> Homo sapien
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542

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469

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238

<210> 118<211> 415<212> DNA<213> Homo sapien
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415

<210> 119<211> 577<212> DNA<213> Homo sapien
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577

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207

<210> 121<211> 246<212> DNA<213> Homo sapien
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246

<210> 122<211> 406<212> DNA<213> Homo sapien
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406

<210> 123<211> 596<212> DNA<213> Homo sapien

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240ctgctacagc atgggctcct aaaacctata aactctcctt accattcccc cattttacct
300gtcctaaaac cagacaagcc ttacaagtta gttcagaatc tgcgccttat caaccaaag
360tttttgctat ccaccccggt gtgcaaaacc catatactct tctatcctca atacctccct
420ctactaccca ttattctggt ctggatctca aacatgcttt ctttactatt cctttgcacc
480cttcatocca gcctctcttt gccttcaact agactgaccc tgacacccat taggctcagc
540aaattacctg ggctgtactg ccgcaaggct tcacagacag cccccattac ttcagt
596

<210> 124<211> 255<212> DNA<213> Homo sapien

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120ttatttttctt ttgtgtacca gaatacttat aattctttga gcctcccaga aattggaagc
180taataaaagc aactcaagtt tccttcaaaa aaaaaaaaaa aaaaaaaaaa aanttttnc
240cccccccnng ggggg
255

<210> 125<211> 332<212> DNA<213> Homo sapien

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120aagaaaaagaa gactctgacc tgtactcttg aatacaagtt tctgatacca ctgcactgtc
180tgagaatttc caaaacttta atgaactaac tgacagcttc atgaaactgt ccaccaagat
240caagcagaga aaataattaa ttcatggga ctaaatgaac taatgaggat aatattttca
300taatttttta tttgaaattt tgcgtattct tt
332

<210> 126<211> 405<212> DNA<213> Homo sapien

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120agtattccca tggcctgggc cagccaagag caggggactg tgcctcagga gcaggcttgg
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240aatgttgggg gcggggtttc ccttgagtaa gtgtgtgtga gggagtgtgc ctgtgatggt
300gattttggct acgcaagtgt ctgtttcctt tctcctcctg ctctgccata gatgctgagg
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405

<210> 127<211> 582<212> DNA<213> Homo sapien

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120atgcttttaac atacaatatt cttgttgaaa tctcttcaaa gagcacagca tgtaaaagcac
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360gttcagaact gtcgggctat tcaggctgtc ttcttggtgc aagctccttg gaggtcttgt
420aaattgatct tcgacctgtt gtgaatcatg tgacttttga caagatgtcc tgctgccaat
480gtgcccataa gtgacaattc cccagccatt acggtcccac acacaattcn ggcaagctgc
540cnggcatttt ctccaggaat atctttgcat gtccttgtaa ca
582

<210> 128<211> 317<212> DNA<213> Homo sapien

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120gtgtgtgctg caccacagca cggggagggg gcacgcancg acaccagag aaggacagc
180cccaccatg tcaacagcag gcaacctgtg ttttcatttc aagtgggata cagtattttt
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317

<210> 129<211> 582<212> DNA<213> Homo sapien
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120tcacgtctc cagggccctc ctggcccccg gcccggaac gtctgggtca gtgaggtccc
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420ctcatcttcc ttagcngct gtcgtgcttg cntgaaaact ctgcncttg tctctgccc
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<210> 130<211> 116<212> DNA<213> Homo sapien
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116

<210> 131<211> 198<212> DNA<213> Homo sapien
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60tcaagtactt ctccagtctc agcctctgag tagctgggat tacaggcaac caccatcacg
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198

<210> 132<211> 308<212> DNA<213> Homo sapien
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60gcagagctca tgtgacttct agacgtggtg acttaaaaaa tggccttaag gctgcagagc
120cagccactc tgcctacaaa aagagtactt agtgacatg actgtaagaa acaattgtaa
180aacctcatct agaaatcaga aagcttctaa tttctataga aatgacacct cctggagcc
240gagagacaat ctgttggtga tttgaagga caggcaagac caacactgta tttagttcca
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308

<210> 133<211> 262<212> DNA<213> Homo sapien
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120acactcatct gactcattct ttattctatt ttaagtgggt ttgtatcttg cctaagggtg
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262

<210> 134<211> 343<212> DNA<213> Homo sapien
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120tctcaaaaag aaaagaaaag aagactctga cctgtctctt gaatacaagt ttctgatacc
180actgcactgt ctgagaattt ccaaaacttt aatgaactaa ctgacagctt catgaaactg
240tccaccaaga tcaagcagag aaaataatta atttcatggg actaaatgaa ctaatgagga
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343

<210> 135<211> 317<212> DNA<213> Homo sapien
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120ttcgtactga gggacacaga cacaggggcc ctctcttca ttggcaagat tctggacccc
180aggggcccct aatatcccag ttaatatct caatacccta gaagaaaacc cgagggacag
240cagattccac aggacacgaa ggctgccct gtaaggtttc aatgcataca ataaaagagc
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317

<210> 136<211> 159<212> DNA<213> Homo sapien

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60gaggaagaga gccactaca gccgccgag cggccgcttc ttgtccgtct tttctcggg
120cgccagcttc ttatcgcgct cgccagcatg cttctttgg
159

<210> 137<211> 264<212> DNA<213> Homo sapien
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120tccattcccc tgggtgtgaa ggcccaggat ggcataacc accctgggtg tgtcaaaatc
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264

<210> 138<211> 263<212> DNA<213> Homo sapien
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120actcgcaatt ggttctgaaa ttagaacgtt caccatcgtta cttaaaatct taggggcatg
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263

<210> 139<211> 459<212> DNA<213> Homo sapien
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60aatccagtgt gcagtctgat gaagtctggg tgggtgtggt ctacgggctg gcagctacca
120tgatccaaga ggtaatgcac tccctttccc atctctncac catctgtatc ctggcccaga
180aaacttcttc aaccacaaaa tttcttcaag gcataacca atgccatctt gtccgtctat
240aaagcctccc atttttccct ggtatgcatt ccagctcctg ccttcaggct tctgtctgtg
300gggtcatagtt atctcctcca cttgtctgga gctccttgaa ggcaagact ctactgcctc
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459

<210> 140<211> 576<212> DNA<213> Homo sapien
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120tggcacttcc ttggggacac tttctgca aacataatc agaggggcag gagctggctg
180tgggtgagag aggtccagca aattcctgtc tgcagaaggg ttctgaacac caccgcctg
240cagcgtgctg gaggaggat tcccttttct ctcacagcaa ttctgaccag aaacctatgc
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420catgcagcct gctggattct gaaagactct ggagacaggg atgtctccca atgagctggt
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576

<210> 141<211> 386<212> DNA<213> Homo sapien
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60aacacataca ctccaccta caggaagaaa tgccacact atttctatat attcgtcat
120ttcttagaat gggcaaacac cttttgctaa aagctatata cttttccact cttttcataa
180taaacacttg atgcattcta tccgtcacat tatthaatca gggacaaagt acctatatta
240tatgattcca aattgtgtga ggaaagtata aggctaacc tgaaaaataa cttagcact
300atgttcattt tcaggtotta gggaaaataa catccaataa attaatcag tatggcttac
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386

<210> 142<211> 227<212> DNA<213> Homo sapien
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120atcggtttca tctcgcctt agcatgattt atctacact ccaactcatg agaccacaa
180caaatagccc ttctaaacgc taatccaagc ctcacccac tactagg
227

<210> 143<211> 246<212> DNA<213> Homo sapien
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60aggcagcctt gtgatcccca cagctcacag gtgagaggct gtcataacct ctccatgac
120tggaagancc ttgnncttgg gacccgacac ctatggnntt tggcccnngg agggagaaac
180ggtgccacag gagttgtctt aagaggacaa ggcntgcacg gtotgagatc agaggtgtgtg
240acgtgg
246

<210> 144<211> 318<212> DNA<213> Homo sapien
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60tgatgacat cagagacagg aactctaacc ttccctatga acatccatgg cactgacagc
120attcagggga acccttcanc tattaaaaan nontccaaca anaactcctc acccttcgtc
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318

<210> 145<211> 295<212> DNA<213> Homo sapien
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120aatgccaagc gttccccaag ctctttntta nccctngggc naagganggg gcctaattng
180ccaaggctgac ctgaggggcc aggacaagat agcctctgct cagaccaacg taagtgcctt
240tgagactcag gttgtgtgtg ctgtccaggc cctggcagat gactatctgg ctgag
295

<210> 146<211> 147<212> DNA<213> Homo sapien
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60agtctttttt tgcactgttg aaattgggct tggcactcaa gtcaaagatg aacatcgga
120taacaaacat tgcctctnc aaaaagg
147

<210> 147<211> 69<212> DNA<213> Homo sapien
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69

<210> 148<211> 671<212> DNA<213> Homo sapien
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540catctnttct gcagcannga taacacgcta ctgacctgc ccnggcggcc gctcnaang
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671

<210> 149<211> 401<212> DNA<213> Homo sapien
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401

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221

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142

<210> 152<211> 626<212> DNA<213> Homo sapien
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626

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143

<210> 154<211> 141<212> DNA<213> Homo sapien
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<210> 155<211> 152<212> DNA<213> Homo sapien
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152

<210> 156<211> 335<212> DNA<213> Homo sapien
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335

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420ctcgaccctt atgcttctgc ttttcttga tggtttcgac agtaacattc tttcctttct
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551

<210> 158<211> 339<212> DNA<213> Homo sapien
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339

<210> 159<211> 385<212> DNA<213> Homo sapien
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385

<210> 160<211> 147<212> DNA<213> Homo sapien
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120taaactgagt gtgnggcat gcnctt
147

<210> 161<211> 176<212> DNA<213> Homo sapien
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60gactaaagaa ccagccaaag ccttaaattg tgcaaacat actgttgcta tgatgtaact
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176

<210> 162<211> 148<212> DNA<213> Homo sapien
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60gctatctgaa gactgttga tatcagagtt ctttgacagt tcacattttt taggttocac
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148

<210> 163<211> 237<212> DNA<213> Homo sapien
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120ccgtctgaac tattctgncc ngcattantc taagtntaa tgggccctcc attccctacg
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<210> 164<211> 337<212> DNA<213> Homo sapien
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120acagtacact ggcaactntt naaactnttg nttcttgggg gcttnttcc aataacatca
180cttgatgcaa ctgggaacct ggtatttgc aatgcgggag gagccccaa catcgtgact
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337

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220

<210> 166<211> 739<212> DNA<213> Homo sapien
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120agctcctgac ncaaggaatg gagggctcnn acctccnng ggggaaaana cnggagtg
180nctcncntcc ttgcctgggg gagnaacaaa anaaagnctg gctttcttgt ttttgagg
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360nggtaaacca gcagcttttc cttcganaaa gaaataccat caaantacct tctaaaagct
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739

<210> 167<211> 290<212> DNA<213> Homo sapien
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120ttttattaatt ctatttaatn aaggaaaaaa acnttnagaa tccataangt ttcagtttat
180tttttagttta ctactagggt gagatagcac attacatact tttactatca aatattattt
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290

<210> 168<211> 250<212> DNA<213> Homo sapien
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120gtgaagaaga aaaacnaaga agggaaaggg aaccacaatt tatntttggg agtttttact
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250

<210> 169<211> 146<212> DNA<213> Homo sapien
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146

<210> 170<211> 292<212> DNA<213> Homo sapien
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120gaaaaagaaa ggattctota ttcccctgnt ttttttttctn ctntatgga cnatatagtt
180tctttttgta agatgcattc attctgacta ttcttaccag cttttattct tactagcatt
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292

<210> 171<211> 151<212> DNA<213> Homo sapien
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151

<210> 172<211> 131<212> DNA<213> Homo sapien
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131

<210> 173<211> 90<212> DNA<213> Homo sapien
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<210> 174<211> 472<212> DNA<213> Homo sapien
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472

<210> 175<211> 752<212> DNA<213> Homo sapien
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360caaacaaagt atggaacagt tgttggccta tgtaaattga aatgtaattg accatittat
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752

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224

<210> 177<211> 294<212> DNA<213> Homo sapien
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294

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142

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180ttcaggcacc agcatccacc ttggctctgc cagtggatcc cctgcgggtc ggctgggacg
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366

<210> 180<211> 104<212> DNA<213> Homo sapien
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393

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311

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277

<210> 184<211> 322<212> DNA<213> Homo sapien
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322

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358

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161

<210> 187<211> 408<212> DNA<213> Homo sapien
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408

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134

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158

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114

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147

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214

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296

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586

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492

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409

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55

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339

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375

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278

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240cgtagaaaac acagacgaga cctactgcat tgataacgaa gctctctacg acatttgctt
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346

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<210> 212<211> 222<212> DNA<213> Homo sapien

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357

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634

<210> 216<211> 456<212> DNA<213> Homo sapien

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456

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120gtacgtgcc gcggaaatgc tccgctagca atcgcacatc cgtgccaag gaccacgcat
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233

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370

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332

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120acaccactgt ccccccacgc cgtctggtca aaggtcaggt cagcatttcc ggtgatgta
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240

<210> 221<211> 381<212> DNA<213> Homo sapien
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381

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636

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235

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368

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221

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374

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317

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330

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508

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216

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412

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214

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176

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526

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411

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120cgacagggtt ttcccatcat ctttcacggc gtaatgggca aagatgagcg tgaaggcaac
180agcccatcct tcttcaacc tgaagaggct gccacaagtg acttctacc tgaagctgct
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319

<210> 241<211> 97<212> DNA<213> Homo sapien
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97

<210> 242<211> 190<212> DNA<213> Homo sapien
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190

<210> 243<211> 376<212> DNA<213> Homo sapien
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120ccagtaaaagc aaactcaaga ttgagcctcc atgtaatgaa ttggggtaaa gaaaaaacat
180gcaggtcaat aggttaggtt aaaaaggtt gttcacacat ttatgacagc aggtcctnaa
240ctgccaacac ctctaaccat ctgattaggt ttctatgagc caagtcttac atattccatt
300catcatgacc ttttagtcaa tgtagcaaca gggattccaa cattttgcta aggaatggc
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376

<210> 244<211> 405<212> DNA<213> Homo sapien

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120caccatgaa ctcaccattc caaataactt aattggctgc ataatcgggc gccaaaggcgc
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405

<210> 245<211> 312<212> DNA<213> Homo sapien

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120aggggtgaga ggttaaagga gccacttatt agtaatgttg atagtagaat gatggctagg
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312

<210> 246<211> 634<212> DNA<213> Homo sapien

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240tctggcaaga taagtaatta gcacagatta ttgtcactca ctgcaactca gccttgagg
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420ggttagaggt agcagaggct gggacagaac ttgtctgttc tgcccccttc caccctcct
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634

<210> 247<211> 325<212> DNA<213> Homo sapien

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120gcactgtaat ttagataaaa ttactgtgtc tctactgtga ttacatgcaa aatccacata
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325

<210> 248<211> 638<212> DNA<213> Homo sapien

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120actaatgaag gctaagcaga atagtctgag tttgctgaga ctaaagcagg gatagtgtg
180aaaagttttc ctttactagc tgggacacat tccccctttc ttttcnaaga ggaagaacat
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480ncagcagact gggtttctat tagaacaagt atcagcaagg tcatgtagac ttgtagaaac
540ttttgccttc tctgtacca gacagatcat tgtccagacc tgccccggcg gccgtcaan
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638

<210> 249<211> 178<212> DNA<213> Homo sapien

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120ccctcctggg tctccacctc atgctctggac aggacgctgt ggcctgtccg ggccttgg
178

<210> 250<211> 477<212> DNA<213> Homo sapien

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360cngggtgact gggtcnctgc ggtttgact cactgagttc tggnttccac atacatnggc
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477

<210> 251<211> 561<212> DNA<213> Homo sapien

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120tcccgcctcct aaggcaggaa gatggtggcc gcaaaaga cgaaaaagtc gctggagtcg
180atcaactcta ggctccaact cgttatgaaa agtgggaagt acgtcctggg gtcaagcaga
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561

<210> 252<211> 284<212> DNA<213> Homo sapien

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120gtcagngttg agtcttattt gttttatttt gctcatagtg actcttcagc agtgcaata
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284

<210> 253<211> 656<212> DNA<213> Homo sapien

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540ctttaacatc gaggatggaa cttngncgcg aacactctaa ggcgaattcc accnccctc
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656

<210> 254<211> 190<212> DNA<213> Homo sapien

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120tgagattcag aacacaacct acctgtggtg ggtaaataat cagagcctcc cggtcagtc
180caggtgcag
190

<210> 255<211> 446<212> DNA<213> Homo sapien

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120ctccacca gaaaatgaga attaaaaaga atttgtcaaa ctatctttaa taatgccct
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240gaccctgac cccgttgatt tccgtccgct aggttgctct ncccatggcg ttttgctggt
300tatganattt gcggaacgcc gtggtggggg gtaaaggga ccaaactcg gccgcgacca
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446

<210> 256<211> 315<212> DNA<213> Homo sapien
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315

<210> 257<211> 524<212> DNA<213> Homo sapien
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240aggtagacaa taaatcagt actaggggt agccctgaga tatgggcagt tcgttcagca
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360aagttctcga agatcccatc ngtagccatc caggagagat tgaagctttt cgggagttat
420gtcagaaaca ttttaagttt caatttcagg ttcttttgct gtggaggact tgcccgggag
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524

<210> 258<211> 261<212> DNA<213> Homo sapien
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60caaggcatga gagggcaagt ttgttggtga cagatctgtg cctactttat tactggagta
120aaagaaaaca agtttcattg atgtcgaagg atatatacag tgtagaaat taggactgtt
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261

<210> 259<211> 190<212> DNA<213> Homo sapien
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120attatataca tacatgaaac tgcaatttta tggcattcta agtaactcat ttaagtacat
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190

<210> 260<211> 692<212> DNA<213> Homo sapien
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120tgatgaattg ggaagagagc aaaccagaaa tggctttatt ttctcccttg gactaatttt
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660gaacttggtg ccaaacttgg cgtaactctgg gc
692

<210> 261<211> 356<212> DNA<213> Homo sapien
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120tataaaaaata ctgggaaaaa aattgataaa taacaggtaa gagaaagata tttctaggca
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240gatcatgctt gttcctacag tattgcgggc cagacactta agtgaagca gaagtgtttg
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356

<210> 262<211> 366<212> DNA<213> Homo sapien

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366

<210> 263<211> 389<212> DNA<213> Homo sapien

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120gtgaagangg tcattattcg agacagccct gttctcctcg tcacctgca gtgtaacctc
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389

<210> 264<211> 409<212> DNA<213> Homo sapien

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409

<210> 265<211> 161<212> DNA<213> Homo sapien

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120tgacaccatc atgtaaggag ctggttgacc tcatgaccg c
161

<210> 266<211> 455<212> DNA<213> Homo sapien

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455

<210> 267<211> 261<212> DNA<213> Homo sapien

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261

<210> 268<211> 111<212> DNA<213> Homo sapien

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111

<210> 269<211> 289<212> DNA<213> Homo sapien

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289

<210> 270<211> 538<212> DNA<213> Homo sapien
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538

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120gcacctctaa gatactgatg gctctgcaga ggaccattc attgcttctg cttttgtctg
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220

<210> 272<211> 238<212> DNA<213> Homo sapien
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238

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504

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360cttcacagggt tgcctatccc tcgtccag
388

<210> 275<211> 344<212> DNA<213> Homo sapien
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418

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758

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392

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88

<210> 280<211> 588<212> DNA<213> Homo sapien
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708

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227

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150

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232

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663

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<210> 291<211> 360<212> DNA<213> Homo sapien
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360

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174

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304

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208

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410

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<210> 305<211> 434<212> DNA<213> Homo sapien
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146

<210> 307<211> 548<212> DNA<213> Homo sapien
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353

<210> 309<211> 590<212> DNA<213> Homo sapien
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120cggggccccg cccccaaat tnggtggaaa atngggaaant tttttttt tggcccaana
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240gggccaagg ggttccctt gcggttcgta accgccagt ccgatacaac ctgccccttg
300canttgggtt nggggtcaca ctgtgtgttc cttttccaa tgaaaaggg ctttgttan
360gaaccagca agttattctg aagaaagaaa aattgtggtt aagggaatgg tactgaggca
420ttgactctgg agaagccctt gctggatcta gtctttctna tgcacatcaa gaatctaagg
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590

<210> 310<211> 318<212> DNA<213> Homo sapien
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120ttggcctcaa acaatcctcc tgtttcanto taccaaactg ctgggattac aggcattgagc
180cccctgccct gtaattttat ttacctcng cccgggaccn ccctaanggc gaattccagc
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318

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120atttctgag cgtctgagat gttagtatta gttagtttt ttgtgagtgt taggaaaagg
180gcatacanga ctagggaagca catnaaggaa aatgattgtt aaggcgacc tgcccgggc
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120ctaataaat atgttgattc atggctataa taaagcagga gcaattataa aatcttcaat
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225

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120ccccctccc agaagaagtg gatgaaacca gtgctgaaga tgaagggtgc tctcagagga
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248

<210> 314<211> 345<212> DNA<213> Homo sapien
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120caatcttaat gtctcttcat aatactttta taatacatta agcctcttgt ctacatattt

180ggagagaata tgactttact agcagagaaa tacaatatat ctgtgtact ggactgtaaa
240atatatgtat gaaataaaat tagttccatt tggctttcta gtatattaaa gtgctatctg
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345

<210> 315<211> 413<212> DNA<213> Homo sapien

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120acaaccagtc agagtactcg gtgggttcag aggaggagga tgaagacttc gatgaacgto
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300aggctttcct caatgctgtg atgcgctggg gtagccacc acaggatgcc ttcaccacac
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413

<210> 316<211> 88<212> DNA<213> Homo sapien

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88

<210> 317<211> 147<212> DNA<213> Homo sapien

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120aagacgagaa aactgaggaa aactcag
147

<210> 318<211> 299<212> DNA<213> Homo sapien

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120atttatatta aaaaatagtg caaaatctca acatttatat aaataactct aaaccctgac
180tttgaattt ttttctttac aaggaataac acactttctg acttggcact caaaaattgc
240catttttttc ctcttctagt tcagaaaaca actttttttt ttaatataga cctcgccg
299

<210> 319<211> 100<212> DNA<213> Homo sapien

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100

<210> 320<211> 325<212> DNA<213> Homo sapien

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120ctctacagaa atacaaaat tagctgggct tgggtgtggc atgtgcctgt agtcctagct
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325

<210> 321<211> 80<212> DNA<213> Homo sapien

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80

<210> 322<211> 86<212> DNA<213> Homo sapien

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86

<210> 323<211> 244<212> DNA<213> Homo sapien

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120cccatcgta gatgagccca ctcatggaca aatgccctac cttgtgtgcc atattcagct
180aacttttttc ctgcaatgag atcaggagtg aggtggatcc tttcaccctt ctctccacag
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244

<210> 324<211> 344<212> DNA<213> Homo sapien
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120atcctatagc tgccaagccc ataactcaga cactggcctc aataggacca cagtcacnac
180gatcacagtn tatgagccac ccaaaccctt catcaccagc aacaactcca acccctgtga
240ggatgaggat gctgtagcct taacctgtga acctgagatt cagaacacaa tctacctgtg
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344

<210> 325<211> 255<212> DNA<213> Homo sapien
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120gactaccatt aatcccagga gggccaggag gaccttgagc accagcgtgt cctgagggtc
180cagggttctcc tctttgtcca ggggcaccat ttgaaccagg agaccctgca ggtccaactt
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255

<210> 326<211> 335<212> DNA<213> Homo sapien
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300naaaagggnn ggggatcntg gaggctttac tttaa
335

<210> 327<211> 295<212> DNA<213> Homo sapien
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120tgtgtcaaga gagaaaatat atatctaata caaaaaagta gccaggcatg atggtggcac
180acgcctgtag tcccagctac tcaggaggct gaggcacaag aatcgctga atccaggagg
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295

<210> 328<211> 417<212> DNA<213> Homo sapien
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417

<210> 329<211> 483<212> DNA<213> Homo sapien
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420ggtcagccct gtagaagtct ccacgtgaac caaagtnata accaccaccc gnttacacac
480ctc
483

<210> 330<211> 358<212> DNA<213> Homo sapien
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120ccagtggtcc atttaaccat ttgatgaaac attattttta tgacttataa aggatagtag
180agnatactga aattccactt aaatactgaa atattctact aatgacatt gttttgtcta
240aatttccctc agaaaaatct gttagcattt cttaaaagtc cctcagattt gagggaaatt

300ctaaattagg acagntttct ctccaaataa atataaatga tcttgagtat ttttgttt
358

<210> 331<211> 306<212> DNA<213> Homo sapien

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60gaataaaact caaaacagga cttcatgttt tatggaaatt atatttaacc acttcaacat
120tgtaaatctg gataactttt aatatctaaa ctatataaga aagtaaaatt taacatgtta
180atatggataa tttttttatt ttgtacott gatgacttaa atgtaagcaa caaagtgggt
240aaacacatac ccatctaaat tttttatag cttccatgt taaactataa gtaaataatt
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306

<210> 332<211> 251<212> DNA<213> Homo sapien

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120gccgttgagc tgtccgttca tgttgggtgct ggtgtgaggaa agtggcctta cgaaggacca
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251

<210> 333<211> 579<212> DNA<213> Homo sapien

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540angctttgac aggaatggac tggatcacct gactccacg
579

<210> 334<211> 534<212> DNA<213> Homo sapien

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240ggataacatg ggggttgatt agtgaccaca gttatcagaa gcagagaaat gtaattccat
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360cccacaaact ttaattttgt taaatttata tggntttgaa atagaaagta taagttgcta
420ccattttttg ataacattga aagatagtat ttaccatct ttaatcatct tggaaaatac
480aagtcctgtg aacaaccact ctttcaccta ccagcatgag ggcaaaagta aagg
534

<210> 335<211> 282<212> DNA<213> Homo sapien

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60tgatgccagc atttgcatcc cgggtcccat cacattcatg cccaatggat gctgcaagac
120ctgcacccct cgcaatgaga ccagggtgcc ctgctccacc gtcccgtca ccacggaggt
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282

<210> 336<211> 193<212> DNA<213> Homo sapien

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120tggtgggtctg ggggcagtgc cctctggccc ctctctagca ccaaaaggga ggaattgggg
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193

<210> 337<211> 341<212> DNA<213> Homo sapien

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341

<210> 338<211> 239<212> DNA<213> Homo sapien
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239

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120cagttgatta ggggtgcttag ctgttaacta agtgtttgtg ggtttaagtc ccattggtct
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222

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314

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180gcctgccaga ggccaatgc tggaatggca ccatcattca catcagaact gcagcccctg
240gaaaagaaga gacagccata gacnaggagc cagagtgggg gcagactgg
289

<210> 342<211> 356<212> DNA<213> Homo sapien
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120taccagagca tcaagtctct gcagcaggtc attcttgggt aaagaaatga cttccacaaa
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356

<210> 343<211> 472<212> DNA<213> Homo sapien
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472

<210> 344<211> 446<212> DNA<213> Homo sapien
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120cccatagttg ctaacaatcc tatttaacca ctaagaaagg atttacaaca ataaaagcta

180tgtacatagg aaatatcagg agagtagaaa gtgctaacca gaggtaccca gaaaagcttt
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<210> 345<211> 197<212> DNA<213> Homo sapien
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 197

<210> 346<211> 499<212> DNA<213> Homo sapien
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 360ccgntaangg ccaattctca aattccatca cnaactggcg cccgnttcga ncatccatct
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 499

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 480caccttaagg gcgaattcca ncacacttg cggccgntct agtggatccc aactcggnc
 539

<210> 348<211> 69<212> DNA<213> Homo sapien
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69

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72

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522

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76

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527

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120tcttcacggg ggagacctgg ttggggtgct cacagaggat gttttccggc ttaggtccc
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<210> 498<211> 310<212> DNA<213> Homo sapien
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319

<210> 520<211> 326<212> DNA<213> Homo sapien
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120aggggtccag cctcgttga agaggaacag cactgggag tctttgtgga ttctgagcc
180ctgcccattg agactctagg gtccagtga tgccacagcc cagcttgcc ctttccttc
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326

<210> 521<211> 509<212> DNA<213> Homo sapien
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120ggggaaaatg ctattctgtg ttttgaaaa gaagaaatag tgccgtccta tttatttcta
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300caagagctta cnaaaactaa gtggnatttg gtttttnac cccctggaa nacctantn
360aaaaggaaag gccctccaat tttatttggc atgcttcaac cataaagaca tttntggtt
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509

<210> 522<211> 343<212> DNA<213> Homo sapien
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120agacgactac caaaccccaa gccacatctg ccccgcccc cgcgccaaag caagcttcc
180tgtttggaac acagaacacc tcaccttcca gcctgcgc ccctgtgca tcttcagcat
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343

<210> 523<211> 369<212> DNA<213> Homo sapien
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60caacaaaaag atgtatgagg agttcaagca gctggtctg gaggacgcca aagaaggcta
120cagataggt ttgagtgcc ttttctgata ctacagttat ggctggaaa agaagttccg
180gctggacata ttcaaggatt ttcaggagga aacggtgaag gactatgaag ctggaagag

240ccagagttgg atctgagtga ggacctcggc cgcgaccacg ctaagggcga attccacaca
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360ttccctggg
369

<210> 524<211> 353<212> DNA<213> Homo sapien

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60tcattctgga tgatgttctg gatcagcagg gatgcattgg ggtatattat ctctcgacca
120ctgtatgcgg gccctggggt agcttgttga gttcctatta catatcctat aatttgacgg
180ttgccatcca ctctttcacc tttgtaccag ctgtagccaa aaagatgctg gggcagattg
240tggacaagta gaagcacctc ctccccctct gcgacattga acggcgtgga ttcaataatg
300agcttggcag tggngggcgg ggttcnnaaa ggtanaaatg aagggtgggna ccg
353

<210> 525<211> 272<212> DNA<213> Homo sapien

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60acactggggg ccagatcct ggtcattccc cacaggctct aataaagggt catggaagga
120aacctgtttc ctaaggtagg gtgggagtgt gtgtgagtgt gtggggggga gaggggtgaga
180gtgagtgtgn gcgtgtgtta ntgtgtgtgt gtngtgnagg agcaggagtg actgggnnct
240gagtttang agtngggaag agganggaga ga
272

<210> 526<211> 653<212> DNA<213> Homo sapien

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60cagagcccaa cttctctgtg gcctatgcca acatgtgccg ctgcctcatg gcgctgaaag
120tgcccactac ggaaaagcca acagtgactg tgaacttccg aaagctgttg ttgaatcgat
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240agatggatga agctgctccg cagangaacn angacgcctg aaggaagact ggaanagctt
300tggacatacc ccggggggct nttttgggan atttaanttt ttgggaaagt tgttcaacct
360gaanaantta canaggcaat aatcngact tgtggggcaa actgcttaan aacctgatg
420aanaagtcct ttgagngcct ttgtcgtctg ctcccccca ttggcaanaa acctggactt
480tnaaaaaagc caacccccaa tggatcaatt ttccaccn atggaaaaaa tntttaagaa
540aaaaaactnt tccccttccc tttttctca ggaantnttg attncaagga caattggggc
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653

<210> 527<211> 223<212> DNA<213> Homo sapien

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120ctttcttctt cttcttctt aaagacattt aagctaaagg caactgtac ccaaatctc
180aagacacaaa catgacctat ccaagcgcat taccacttg tgg
223

<210> 528<211> 404<212> DNA<213> Homo sapien

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120aagaagcctg ttctgtccag cttcagtac acaagctgct ttagctaaag tcccgcggt
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240gagcaggaat tcagctccta ttctccaatg gagagatctg gcctcanctt gggctagaga
300tgccaangac ctgcccgcgg gccctntaaa ggggaaattc nancacctng gggccttct
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404

<210> 529<211> 357<212> DNA<213> Homo sapien

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60tgggaatgac ttgagtttga gtacagatat tcgacaccaa aaaagtcata ctacaatgaa
120ttcctatgaa tgttatcaat gtgggaaagc cttctgccga agttcatccc ttattcgaca
180tcagatcatt cacacaggag agaaacccta taaatgcagt gaatgtggga gattcttcaa
240ccgacgtaca aaccttacta agcatcaaaa acttcatgct gaagcaagg acctgccccg
300ncgggcgctc caaangngna atttcngccc cctggggggg nggtcnttag gggaacc
357

<210> 530<211> 179<212> DNA<213> Homo sapien
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60gacctgggtg ttcctctttg tgggtggcagc agctacaggt gtcgagtccc aggtgcagtt
120gggtgcagtct ggggctgaag tgaagaagcc tgggtcctcg gtgaagggtct cctgcaagg
179

<210> 531<211> 288<212> DNA<213> Homo sapien
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60ctgttaagaa tgtctaatat gcaaagtatt ggaaagcagg catttttacc aaagagcaat
120gtcactataa cttatgaaca gaaagttgtg aaatataagg gtactcatgg aaaccagtga
180agagaggaaa caccggcaat tgttcaacac ggaacagtga gcaggtactt tgggagtaag
240tccactgtaa tagtccagtt tgggtctcag atctgaggng atgtctgg
288

<210> 532<211> 320<212> DNA<213> Homo sapien
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60ccataatcaa tcctaccaat gctgacattg accttaaaga tgacctagga aacacgctgg
120agaagaaagg tggcaaggag tttgtggaag ctgtcctgga actccggaaa aagaacgggc
180ccttggaagt agctggagct gctgtcagcg caggccatgg cctgcctgcc aagtttgtga
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320

<210> 533<211> 578<212> DNA<213> Homo sapien
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60aacatcagaa agcctgggct ttgaacctga acggttttga ttagaggaa gccaaagatcc
120ttcggctcag tggaaaacca caaaatgcgc cagagggtta tcagaacaga ctgaaagtac
180tctacagcca aaaggccact cctggctcca gccggaagac ctgccgttac attccttccc
240tgccagaccg tatcctggat gcgcctgaaa tccgaaatga ctattacctg aaccttgtgg
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360antttgggac atcctgagct tttgaaatgg acacctgggg aatttatctc tgggctgata
420aaaaggaact ctgtctggc accacatgtg agtnacttgg atggagacaa acggttcaat
480tangcttcc atggtctact gacantttct gcatgtntct tgactccgcg cgtagnnacc
540ccattggcgt ctangtctact ggnactgca antggatg
578

<210> 534<211> 457<212> DNA<213> Homo sapien
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60agttcactac atatgggttg tttgagtttt ttgtgtgctg tatttcttcc tgttttttaa
120tacctggttt tgtacatatc taactctgtt ctcttttggg tgttcagaaa ctggattttt
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240ataggtgttc atactgttac atccaaaaca tttgtcaggc tctctgtcag ctttcatgtc
300atatggnata gaaaccatgg aggtaggccn tccnggatnt tttttttaga aaaaaancgg
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457

<210> 535<211> 394<212> DNA<213> Homo sapien
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120tgtgttctgc ttggctgaga actcgggtgg gccaatgcg acaaggccag atgacatcca
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394

<210> 536<211> 324<212> DNA<213> Homo sapien
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120agagatggct gagagccctt aacctgaggc ggcaccacaa taggcagcaa caactgtgtg
180gaaagctgga tgaactggtc agtagcggaa aatgggaggg ggcactgggt tggcctcttg

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324

<210> 537<211> 314<212> DNA<213> Homo sapien
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314

<210> 538<211> 160<212> DNA<213> Homo sapien
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60aagctcttac agaaggccat gtccgggtct gtgtcactgc ccgagaacac atccctctgg
120ggcagctcca tccgcagggt atcaccccg atcacatagg
160

<210> 539<211> 401<212> DNA<213> Homo sapien
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60caataagac ttctctatat aataatgcct ggccaatgt cccctgtaac atcttacatg
120caggacgtag ggccaattct ttcataaccc acaggcaact gtgagtgtcc tttctcaaat
180agtgaaggta ggtattaaag cacttttggg gggacagatg cacagggtgt gcctctgaat
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300aactgccng gcggccctcg aaaggngaa attccacaca cttgngggcg tncnaagggg
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401

<210> 540<211> 328<212> DNA<213> Homo sapien
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60gatgtgctgg gggcggccac ccgctctcca gaaaggcac agctgaggca ctgtggctgg
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328

<210> 541<211> 615<212> DNA<213> Homo sapien
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360ttttgatant ntgggnagg gcgnacagta ccttcaacag gactgctgac aacccgagag
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480ggaataacca gtggcctagg ggtggagatn tgnaaaagg gagactaccc caatgattgg
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615

<210> 542<211> 448<212> DNA<213> Homo sapien
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60taagnggatg ttttgtnca acttatanaa aaggtaaagg aaaccccaac atgcatgcac
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448

<210> 543<211> 170<212> DNA<213> Homo sapien
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60gatcatcaga tcagtctcaa atgggtttct tggaatttta ttttgacaa tttttatact
120ataccaaact catttgcaat tcttaggttt gttggttaaa acattttttt
170

<210> 544<211> 572<212> DNA<213> Homo sapien
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300cgtccaaggt gtgggcccgtt ggcncctttt ttcaaaccgg gtttttttaa ncctngggcc
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540cgnaccaagc ttggggnaat atggcatact tg
572

<210> 545<211> 70<212> DNA<213> Homo sapien
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70

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427

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359

<210> 548<211> 362<212> DNA<213> Homo sapien
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240gactggtccc aagggtactg caccacagg caatttctg ccatagttag gaaggaaaca
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360tg
362

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318

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120tttttggaaat acatgtgtca aaggctgccc atgttaatac ctttggata aaacggtaac
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555

<210> 551<211> 490<212> DNA<213> Homo sapien
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490

<210> 552<211> 197<212> DNA<213> Homo sapien
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120tataaatcta ggtcttctgg gtcattaagg tattaagctt cagtgtcttt tttttttt
180ttttttttan cccaaa
197

<210> 553<211> 484<212> DNA<213> Homo sapien
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480ttcc
484

<210> 554<211> 200<212> DNA<213> Homo sapien
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60cacatcatag acttcacttc caactccttg gaatgttcat ttctttggct tacaggagag
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200

<210> 555<211> 324<212> DNA<213> Homo sapien
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324

<210> 556<211> 349<212> DNA<213> Homo sapien

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120cctcagagtt ggcctttgaa ccaagtgcc ctggaccagg tcagggccta cagctgtgtt
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349

<210> 557<211> 330<212> DNA<213> Homo sapien

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180tactcagttg gtgtggttgg tacgggaact ggtgaagagt ggggttctgg gagccgatgg
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330

<210> 558<211> 314<212> DNA<213> Homo sapien

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180aaagaagttc ctttccacat aaggcacagg acaaaattaa tcccatttac atattcaagg
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314

<210> 559<211> 321<212> DNA<213> Homo sapien

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180tgtgattcaa acttctgtgt actgggtgat gcaccattg tgattgtgga agatagaatt
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321

<210> 560<211> 235<212> DNA<213> Homo sapien

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120aagattccta ccaccagtta ctttgggcca agtatccaca tccccttgcg tatgggaggt
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235

<210> 561<211> 330<212> DNA<213> Homo sapien

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120gcatggagtc ctgtggcacc cagaaaacta cttcaactc catcatgaag tgtgacgtgg
180acatccgcaa agacctgtac gccaacacag tgctgtctgg cggcaccacc atgtaccctg
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330

<210> 562<211> 348<212> DNA<213> Homo sapien

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120caagcagttt ctttgcgtgg gttactcaag ggcttgtggt tacttgatc tcctctatgt
180gaacttgact ttgaaagaca gagctctagt gtgccagcct gtaagtcct gtaagaatag
240ggaaggcgg aggggggtgg gcagtgacta agggacgaaa acatggggaa aatatttcac
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348

<210> 563<211> 325<212> DNA<213> Homo sapien

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120cctgtgggtg gtaaacaatc agagcctccc ggtagtccc aggtgcagc tgtccaatgg
180caacaggacc ctactctat tcaatgtcac aagaaatgac acagnaantc acaaatgtga
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325

<210> 564<211> 172<212> DNA<213> Homo sapien
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172

<210> 565<211> 203<212> DNA<213> Homo sapien
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203

<210> 566<211> 510<212> DNA<213> Homo sapien
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360agcncntntt aaaaaactan ccgntcagga acttngggcc gnaaccacc cttaggggg
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510

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319

<210> 568<211> 340<212> DNA<213> Homo sapien
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340

<210> 569<211> 330<212> DNA<213> Homo sapien
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330

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120atcttgcatt nncgtatgcc acctntgaac atcaatggct aaaatgttct caaacatagt
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371

<210> 571<211> 342<212> DNA<213> Homo sapien
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342

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314

<210> 573<211> 438<212> DNA<213> Homo sapien
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438

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253

<210> 575<211> 248<212> DNA<213> Homo sapien
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248

<210> 576<211> 272<212> DNA<213> Homo sapien
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272

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509

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287

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455

<210> 580<211> 351<212> DNA<213> Homo sapien
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351

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250

<210> 582<211> 115<212> DNA<213> Homo sapien
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115

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294

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432

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568

<210> 586<211> 345<212> DNA<213> Homo sapien
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<210> 587<211> 116<212> DNA<213> Homo sapien
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116

<210> 588<211> 360<212> DNA<213> Homo sapien
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461

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492

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377

<210> 592<211> 401<212> DNA<213> Homo sapien
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300gatacggcat ctnttggcaa tcagtncagg gncgngctn cagctccan cangatgcnt
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401

<210> 593<211> 377<212> DNA<213> Homo sapien
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240ctgccaatac ctgatttctg tccccatttt ttccactgc aatcatatac ttaggtacct
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377

<210> 594<211> 310<212> DNA<213> Homo sapien
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120agaacctacg cacctacgtg aggagcttag ccagaaatgg gatggactga acggacagtt
180ccagaagtgt gactggctaa agctcgatgt ggtcacagct gtatagctgc ttccagtgtg
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310

<210> 595<211> 434<212> DNA<213> Homo sapien
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180accacttgtg ccgctttatg gtgtagacca ggggcaccag cagagccatc atcatcaaca
240tcttgagccc catgctttt cgatggctgt gctctggctc agatgccag cgcangaagg
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420taangggcga aatt
434

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360ctctggacaa agtcttncg gaacgggagt cctggaatgc cagcattgtg gatgccatca
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600cctccaancca aaangttaaa gataatanna caganagcan cattgnaag ccagcnaact
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740

<210> 597<211> 448<212> DNA<213> Homo sapien
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120cctcnacatc tctgctttgg gggattttta ccttgtctgc acacttgtca ggggagaggg
180gacagcaagg tgggaggttg aagagctttg aggtcagca gcatgtttgt ggcattcggg
240ggacaccatg gccttgggag gctggacang tttttgtgat gtgaaggaca tgcattgggg
300acatggtaag cttggcaagg gctncangaa cgcttnacta anggttttan gacccccacc
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448

<210> 598<211> 363<212> DNA<213> Homo sapien
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180atggcttggg aggcgtgggc gtcgacttgg agggcggtgg tgttggggag ggcgtggatg
240ggcaanacca gntatagaac tcaacgtaca gctcantgag cactgacta aataacangt
300gttcctatgt gccgttgtac acctctccct gtgcgatcng gtgtagtcn acacagccat
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363

<210> 599<211> 488<212> DNA<213> Homo sapien
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120tcaaaacgac cacagtccca cctcagttca aggtaaagcc ggatttcctg ggcgggggtc
180ccacaggacc tctgtagta gcccctgcgc tgtgtgtctg gagcggggcc tcggccttat
240tgaaatggtc caagnagaca gctgcttgtt ggattccagt gcagggaccc tgcgatgttt
300acgtccacac ccnagcccag tgtgggactg ncatttttaa atgggagcgg naaantgggg
360attggacttt taagacgggt acctaaanaa naatttttat nngtaactga agcaacctct
420tttgaaaana actgtattgg nacnggnagg nggaggangg cttggaangg atnaatatnc
480ntntcctt
488

<210> 600<211> 259<212> DNA<213> Homo sapien
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60cgactgctg gagcgggctg ggtaactaca ggaactgtct ctgtgtgata agatttggtg
120attccacttt ataagaaagg aaactgcttt aattaaagca cgtaataat taaaacttca
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259

<210> 601<211> 386<212> DNA<213> Homo sapien
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60gattgcccgtg aacctccggc ccggtgacaa gaccaccttc cagctacagg ttccgagggt
120ggaggactat cctgtggacc tgtactacct gatggacctc tccctgtcca tgaaggatga
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240caacttccng ntgggatttg ggtcttttgg tgataaggac atctctcctt tcttctacac
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386

<210> 602<211> 317<212> DNA<213> Homo sapien
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60gtgccaaaaa agaaagaccc aaaagaaaca aaaacaaaaa ccctaattgt gacactgatg
120gtcagtagcc tgaacagtgg ccaaaggcc actgatcaaa aataaaatag tggctgtata

180tcaatgaaat gaaatccaag aagctttaac cctttggcat cagaaatcca gatttttcaa
240ttatgtaaat gcttatttct tacattgcc aagagtggga gaagtgaatg tgccantgng
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317

<210> 603<211> 378<212> DNA<213> Homo sapien
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60cttcaggcac ctgctgtgcc tccttctcog cagatgctct ggttggaagc ctccctgcaat
120gccttctgta acagcaccag ctggacgttg tcatgaaatg tcacgagttc tgggtgtttc
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240tctgaggagc tccangacct aggtntctcn cttttgaann gatggnacat gcctngatgt
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378

<210> 604<211> 359<212> DNA<213> Homo sapien
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60aaaaagaaag caaagacagg aagtctactt gccactcag taattaacaa agatttatgt
120aaaattccca caaattgatc tcagaatcag aatcagcatt ttntattagc aaaagctggg
180aaatactagt aattgtgctt tatataaagc tgtttgaggc aatctgcata ggaaatatta
240tcaagattca taattgccca gatctgaagg acctgcccgg gcggccnnta aggcgaatt
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359

<210> 605<211> 222<212> DNA<213> Homo sapien
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60gtctccattg gctccaaggg ttctgtgagc cacagaaggt tgtgaaagga gaagacctga
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222

<210> 606<211> 507<212> DNA<213> Homo sapien
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120ctgtctgccag cttatttttc tgggtactgt attttcacat gttaaatgat ctttatatat
180gttgaattaa caaatatttt gagtttctga gaaaaacaa aacatattaa tgggtattgaa
240atgtgttagt agtctggctg tgtgcccata attctgttcg cagcaaaagt gaaagacctg
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360ctaacttggg ngtnngaat gntngcttna tntcccgacn tttttccct gncggngggc
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507

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180tttcgacttt gatggcactc ctaggctcta tcagactgac ccctcgggca cataccatgc
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326

<210> 608<211> 336<212> DNA<213> Homo sapien
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120ggaatgcac ctccagacac agcaggagtc agcgggagg cacagacctg cccctgcca
180ggcagaaaat gggcctctc aagcacaata gtgaccaagt acaattttca gttgtaaaaa
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336

<210> 609<211> 341<212> DNA<213> Homo sapien

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240ttctctcaga ttccacaga gacngttttg aatgttttca aaaccaagta tcacacttta
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341

<210> 610<211> 362<212> DNA<213> Homo sapien

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300ccaattncaa ccccentttc ttaagacctt ggcngaacnc cttaggggaa ttcaacnact
360gg
362

<210> 611<211> 76<212> DNA<213> Homo sapien

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76

<210> 612<211> 614<212> DNA<213> Homo sapien

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120ttttgggtgt aaagtgcctt ttcttgatag agtgttcaat gcacacaaat ccntaataat
180ataacanggc aattcaattt gaagtatgtt agaagggaaat agagacacag gatataaatg
240cataantccg attaactaat cggacatgta aacagttcaa aaataacnaa ctctctctct
300ntctacaatn aaannoctca tgtccgtnaa aatccatttc nttaaaaaaa caaacttctc
360ttcaaantgg gtgtgacccc ggttccaaaa tgtnggattt ggnaaaaacc ctcatttaan
420ttaanagggt antcngaaca aaagtgcggg gctttanctg ctcttngtgc ccttgtnngc
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540ccctaancca gnaccnccn ggccggaaaa angnnccatt tnaccccgca tnnttaggct
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614

<210> 613<211> 338<212> DNA<213> Homo sapien

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120tgtgtgtgt gtgacctcg gtgggtgcca catgctgcta cctgctctcc agtatttttg
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338

<210> 614<211> 243<212> DNA<213> Homo sapien

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120tgtatgtcaa cagaaggat cgtctcttg aagagaactt tactctatcc ttcactancc
180catctttcta nggttactcg cgaccctagn atccccggg attgacactg ccaactgcntt
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243

<210> 615<211> 187<212> DNA<213> Homo sapien

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120ggctgtcctt agagcaagcc ctgcctctg agccaaagga agaaaatgct gagcctgtga
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187

<210> 616<211> 381<212> DNA<213> Homo sapien

ctctgctgct ctgggggatta ctgatcaaa accttccttc cctggctact tcccttctc

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381

<210> 617<211> 315<212> DNA<213> Homo sapien
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315

<210> 618<211> 182<212> DNA<213> Homo sapien
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180gg
182

<210> 619<211> 133<212> DNA<213> Homo sapien
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133

<210> 620<211> 178<212> DNA<213> Homo sapien
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120ccaccaatgt gggggatgaa ggcgggtttg ctcccaacat cctggagaat aaagaagg
178

<210> 621<211> 280<212> DNA<213> Homo sapien
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180ccaccctntgt caacagcagg caacctgtgt gtctatttct agtgggatnc agnatttttn
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280

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311

<210> 623<211> 269<212> DNA<213> Homo sapien
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269

<210> 624<211> 365<212> DNA<213> Homo sapien

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120cttcttcagt cgctccaggt cttcacggag cttgttgcc agaccattgg ctaggacctg
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365

<210> 625<211> 391<212> DNA<213> Homo sapien

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391

<210> 626<211> 489<212> DNA<213> Homo sapien

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420gtttggtgaa ganagaatan tcttctcatg aaattgctan tggtaatcan nctgcgacc
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489

<210> 627<211> 442<212> DNA<213> Homo sapien

tncatagggc natggccctc ngatgntgct cgagcggcgc cagtgtgatg gnatctgcag
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442

<210> 628<211> 316<212> DNA<213> Homo sapien

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316

<210> 629<211> 424<212> DNA<213> Homo sapien

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424

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120acggcatgac cctcacctct atgtaccaga attccaacat ccagatccac cactatccta
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411

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720at
722

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120gttagcgcat gaggacctaa aaaaagctgg attgctgatt ttgtctaata aacaagatgt
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438

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258

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359

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549

<210> 637<211> 645<212> DNA<213> Homo sapien

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645

<210> 638<211> 385<212> DNA<213> Homo sapien

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240cacttgaacc caggagtcgg aggttacagt gagccctgat gggccactgc actccaacct
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385

<210> 639<211> 261<212> DNA<213> Homo sapien

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120tgactttgtc atgtgaaag gctgtgtggt gggaaccaag aagcgggtgc tcaccctccg
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261

<210> 640<211> 303<212> DNA<213> Homo sapien

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120tgagtggtg ggggtggagaa agtaccocgc acctccagt gggcagnagt agacaagggg
180tagcaccaaa cagaaggacc cctccccagc acncacaaca tccaccttca attaccagat
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303

<210> 641<211> 295<212> DNA<213> Homo sapien

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180aatgtgccta ccagattctg gtacaggtca aagaggtcct ctccaagctg agcacgctcg
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295

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607

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446

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120ttgcaactga ccagtgggtc ttcacagggtg cggagaggcc agcttctegg tcttcacctc
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223

<210> 645<211> 402<212> DNA<213> Homo sapien
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402

<210> 646<211> 109<212> DNA<213> Homo sapien
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109

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177

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180ggtgcacagt ggcctcgaac agtggcagga agatgttctc cagcatctcc tggaagtcgg
240

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325

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223

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311

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412

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327

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512

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824

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124

<210> 659<211> 135<212> DNA<213> Homo sapien
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135

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540ggggaattcc cttggaagt gntgggcctt tccntgggt tgtttttcc
589

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543

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168

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192

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102

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300tgtccacttt tgctgtgaac ctacaactga tttacctgcc cggcggnccg ctcgaaaggg
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<210> 693<211> 446<212> DNA<213> Homo sapien

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300gcgggcgctc tcaggcaggc atgactggct acgggatgcc acgccagatc ctcttgatcc
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<210> 694<211> 263<212> DNA<213> Homo sapien

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120actagtgaga cccacatttt gccaccatcc acttcatgtt gacaggagcc ctggtctctg
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263

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120tggaactcat atcaataaac actgttacag caaaaaaaaa aa
162

<210> 698<211> 526<212> DNA<213> Homo sapien

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 526

<210> 699<211> 549<212> DNA<213> Homo sapien
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 120aacctgtttc ctaaggtagg gtgggagtg gtgtgagtg gtgggggga gagggtgaga
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515

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459

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454

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472

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411

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418

<210> 711<211> 526<212> DNA<213> Homo sapien
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362

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307

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503

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341

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411

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 176

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302

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341

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313

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205

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369

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271

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412

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240gaattgagca atcttaccag ggattgaaaa ctgagggtgt gagatgctgg gctgagggcc
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290

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120gtcactataa cttatgaaca gaaagtgtg aaatataagg gtactcatgg aaaccagtga
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288

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120cgggaatgtg aagcgggaag ggtcttacat gctgggtgtc tggggcaagg agactgggga
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246

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300gccgagattt tcagaaatgg ncccatgtga ccaagttctg ctgtttgggt gacagtgtt
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411

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581

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270

<210> 766<211> 449<212> DNA<213> Homo sapien

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449

<210> 767<211> 466<212> DNA<213> Homo sapien

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 427

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<210> 773<211> 294<212> DNA<213> Homo sapien
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294

<210> 774<211> 559<212> DNA<213> Homo sapien

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559

<210> 775<211> 573<212> DNA<213> Homo sapien

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592

<210> 777<211> 372<212> DNA<213> Homo sapien

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372

<210> 778<211> 381<212> DNA<213> Homo sapien

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381

<210> 779<211> 530<212> DNA<213> Homo sapien

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530

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465

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378

<210> 782<211> 430<212> DNA<213> Homo sapien

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430

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364

<210> 784<211> 442<212> DNA<213> Homo sapien

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<210> 785<211> 359<212> DNA<213> Homo sapien
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359

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367

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333

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310

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149

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276

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246

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369

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501

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234

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258

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238

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124

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451

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487

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240ccactgtaat antccagttt ggggtgcaga caagtgtga anaacttctg gaaaagacag
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525

<210> 824<211> 317<212> DNA<213> Homo sapien
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120aanaangaaa aatatanaag tgantcattt nccatngaaa aanggcattt ccagcctcaa
180cntaacctca actagttttt attgcattat tttgaaatg ccaagaaact ggctttggac
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317

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120ctttagtgtt gtgtatggnn atcatttgtt ttgagggtag tttgattacn cattgttggg
180ngnggattan ccngttggtt catnagatat ttncangngg ggatcaatac aggggggaaat
240ac
242

<210> 826<211> 348<212> DNA<213> Homo sapien
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240naccctggc ctnttncccc tccttttana anccccatna tcagcatnch taaaaancta
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348

<210> 827<211> 349<212> DNA<213> Homo sapien
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120agattgctag tctagatggg gcantcttca aattacacca agacgcacag tggnttattt
180accctcccct cctcataaga acttaaaaaa aaagaaaaaa caccctnca aaaaaantca
240aanaatttga ggaaccctt ccaaacagtn cacagttatt aagttcangt ggtcaataat
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349

<210> 828<211> 191<212> DNA<213> Homo sapien
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191

<210> 829<211> 447<212> DNA<213> Homo sapien
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180aactggatct ggcgcttcta ctttgagggc ttctttgacc tcattgctgt ggtggccggc
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300ggaaagaagc tcagtttgcc cngcataagt gccaaanacc atcaccagca tctgtccttc
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447

<210> 830<211> 548<212> DNA<213> Homo sapien
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120cctattcttt ggacataact atgaattttg tatacaatgc acttcatgaa aagttgtggc
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548

<210> 831<211> 183<212> DNA<213> Homo sapien
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60aagatagaac tgggtggttg ggttctgggc agcccatgct tcagccctg caagctgatg
120gtaccgagca tgagactgtg aggtacgggc cccatcacat ggtgctaaca taatctgga
180agg
183

<210> 832<211> 169<212> DNA<213> Homo sapien
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169

<210> 833<211> 351<212> DNA<213> Homo sapien
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120catagtgggt gctgactgtc tctgggttat agttcaccat gatggtctta tatcccatct
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240ggtagacgcc agagccaagg actaggacat gaggtgttcg aaagggtgag tcatgggtg
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351

<210> 834<211> 478<212> DNA<213> Homo sapien
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360gatgaaactg catctctact gcacatgang gcttttnatt tgtanggaca agaanganga
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478

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420t

421

<210> 836<211> 515<212> DNA<213> Homo sapien
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515

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240ttgaaaaaca ccncnactg tcgagtttgc ctacctgagg gaccttntca tnangacngc
300acatncaaaa cattcaang acattaccna ctantcttc acttttaang cctaccctnn
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416

<210> 838<211> 58<212> DNA<213> Homo sapien
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58

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120aaaanttgaa ncttgatcgg tgagtatggg ctccggaaca aacgtgaggt ctggagggtc
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193

<210> 840<211> 468<212> DNA<213> Homo sapien
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120ctgcaaggnn cagctttctc actaggatgg aaaagaagcg tttctgagga acaattcaca
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468

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449

<210> 842<211> 177<212> DNA<213> Homo sapien
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177

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120cca
123

<210> 844<211> 507<212> DNA<213> Homo sapien
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507

<210> 845<211> 434<212> DNA<213> Homo sapien
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434

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317

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464

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561

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428

<210> 850<211> 391<212> DNA<213> Homo sapien
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391

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329

<210> 852<211> 279<212> DNA<213> Homo sapien
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279

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267

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335

<210> 855<211> 348<212> DNA<213> Homo sapien
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240acaaagagac agaagatga aaaagaagaa gagggaggtg gtgggagcgc cgtcatccc
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348

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371

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358

<210> 858<211> 346<212> DNA<213> Homo sapien
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346

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327

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278

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231

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108

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124

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87

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420

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314

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120gtcttgaaga gaggaagaaa aacttttttag aggaacttaa tggtaacata aaccaaactt
180ccactgtatt agtatttgag acaagattac atctatgcat tcacacagct tgtctgtaga

240tctgagagct ccaagggagt ggcccagccc ccattcctct gacttttagcc ttctgaaaag
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353

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331

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690

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432

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378

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438

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327

<210> 903<211> 262<212> DNA<213> Homo sapien
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262

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482

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224

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326

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369

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211

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331

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325

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313

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415

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314

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403

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83

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347

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339

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102

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142

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349

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220

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465

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261

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301

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310

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306

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<210> 947<211> 581<212> DNA<213> Homo sapien
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283

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692

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327

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462

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396

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223

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425

<210> 967<211> 339<212> DNA<213> Homo sapien
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120atagcccttt atttaaaaga gagaagtcc ttttacaag ttattaaatt aattatatgt
180ttaaaagtta aagaaaaaag agctgcagag tatttataaa actgtctttt agaaaaaac
240aagcaagaag accatttgac catatgaatg gaaaaggga gaaagtataa tanaaacttt
300gctagttaaa aaaaaaaaaa aaaaacttgg ntcgnaacc
339

<210> 968<211> 291<212> DNA<213> Homo sapien
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120agtgagaagt caccgcacca ccacgtccct ccgttcctgt tggcaccccc ccacccatcc
180atctgtggcc cccgtgggcc ctgacttgct aaggcgctgg cgggcatagc cttgggagca
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291

<210> 969<211> 130<212> DNA<213> Homo sapien
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120tgccccaga
130

<210> 970<211> 210<212> DNA<213> Homo sapien
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210

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120tt
122

<210> 972<211> 108<212> DNA<213> Homo sapien
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108

<210> 973<211> 313<212> DNA<213> Homo sapien
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120tgtggcagga ctgctgtgaa nactgtatgg acnacgggn agttcaantc ctttntctat
180cattttcgan gcanacangt tctcttgact tcatctacca ggaggacana ccggaccaag
240aatacaatac cactgaaaat acccantgtt nggtagacag ggggaacatc tcancnecat
300cacctnaggc ttc
313

<210> 974<211> 272<212> DNA<213> Homo sapien

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60ggggaaaagg taaaattgga atgtttccag aatctcaca aaaaaaaca acaaaccgat
120gttntaagtg cccaacatga acaaattana accttaaata aagggtcagt ttaatgccaa
180tactagcata ggttcagcac caagcnaat gttattttac tgggtngcct ttttcattct
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272

<210> 975<211> 375<212> DNA<213> Homo sapien

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60cagccccagg cacgaggatc agctcgaggt caccagggaa ccggccaggc ggctcttcct
120ttttggagag gagccatcaa aactcgatca ggaatgtttg gccgctcttg aatgtgcaga
180cgctcgacccc catcagttcc cggccgtgca cagatggaag agtgctgtcc tgtgtactc
240accctcggac agacagagtt ggcccagtc cgcggtgaaa ggaaggttca agtctcagct
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360ccccgcaaag ccagg
375

<210> 976<211> 340<212> DNA<213> Homo sapien

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60cctggcttg gaagggaaga gaaaaagac gcaggccacc tgggggttct gcagtctttg
120gtcagtcacg ctttctatct tagctgcctt tggcttcgc agtgtaaacc ttgcctgcc
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340

<210> 977<211> 429<212> DNA<213> Homo sapien

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120gaagttggct tccatctcag agaagacgc cagctttccc cggagagcag tgacaccag
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429

<210> 978<211> 390<212> DNA<213> Homo sapien

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120ttanaagtga agcttggctt caaaatacaa acactggggg ctttggctca acctttta
180ataaaaaaat tcaatgatgt acaaaaattt gaaagtgtga caatgacaat tatgaaatcc
240tgtgactgaa agtcccctcg agtgcactct gtnggtgcac atgcgcccgc ccacacaaac
300tctggnttgg aaacataaac taatgcaaac cagngctncc cagaagcacc aacagctgtg
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390

<210> 979<211> 372<212> DNA<213> Homo sapien

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120gcaacagaac agtgttcaca gcatcaca ggggtggcatt ggggtggacg ttctgggtac
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372

<210> 980<211> 261<212> DNA<213> Homo sapien

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120tcaaatgttt tgaattttgg aacattttctt gtttctgact ttttagattag ggatactcac
180cccgtaccaa tatttggagt ccttagacat tagattatat gaaaatgatt gattgatagg
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261

<210> 981<211> 266<212> DNA<213> Homo sapien
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120tnttgagaga cttnctcttg gctcccagga ngagggatc cctgactttg acacacatgg
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266

<210> 982<211> 199<212> DNA<213> Homo sapien
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199

<210> 983<211> 344<212> DNA<213> Homo sapien
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180gcgggtgggca natgttttca tanacagcca gggagtaaac acttgttggc tctgtgggct
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344

<210> 984<211> 400<212> DNA<213> Homo sapien
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400

<210> 985<211> 232<212> DNA<213> Homo sapien
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232

<210> 986<211> 347<212> DNA<213> Homo sapien
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180actcattcat ctgctcctgg ctgatccct tggcatccg ggtcaggatc tggttctcta
240cctcattgat ggtcctggcg atggtggtga gcanctgctc ccagcccaca cggatgtgct
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347

<210> 987<211> 439<212> DNA<213> Homo sapien
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120taactgactt cttgaggtaa gattgttctg tcagaaaacc ctctccagc tcccctgag
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240gaagaanatc cttntcaag aagggaaca cgggaaatga gaggtcctg catgcagagc
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360ttcctctggn nnaccactgg cntentagat ntnnagcntg gtggccantc cgnacccttn
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439

<210> 988<211> 256<212> DNA<213> Homo sapien
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256

<210> 989<211> 380<212> DNA<213> Homo sapien
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120tgtnttatgt aaataataaa ctaattgtgg cttgtaaatg attttgtatg tgatcctgtc
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300tcaggggnat cttgtctttt tgttacttnt tttgtgcogt ggtctntcat tgggggtctt
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380

<210> 990<211> 366<212> DNA<213> Homo sapien
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180gagacaaagt gggagccagc gaagccacnc tgetgaacat gctcaacatc tccccctct
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366

<210> 991<211> 302<212> DNA<213> Homo sapien
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120ggccttgccg tgcctccttc ccacgtggt ctttgacntg gaagaggaac tcctgcaata
180acttntatcta tggaggctgc cggggcaata agaacagta ccnctctgan gaggacctgc
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302

<210> 992<211> 569<212> DNA<213> Homo sapien
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569

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360ga

362

<210> 994<211> 501<212> DNA<213> Homo sapien

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501

<210> 995<211> 374<212> DNA<213> Homo sapien

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374

<210> 996<211> 304<212> DNA<213> Homo sapien

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304

<210> 997<211> 344<212> DNA<213> Homo sapien

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344

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542

<210> 999<211> 285<212> DNA<213> Homo sapien

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285

<210> 1000<211> 133<212> DNA<213> Homo sapien
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133

<210> 1001<211> 112<212> DNA<213> Homo sapien
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60attangcaen aatgacatct tnaaaactga ncacnttctg gnaangaaaa aa
112

<210> 1002<211> 273<212> DNA<213> Homo sapien
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180agcgcatgga gccagtggtg ctaaagtcta catactccaa ctgaaatgtt aaggaagaag
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273

<210> 1003<211> 585<212> DNA<213> Homo sapien
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585

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480tanaaangac ctgccctggc ggnccctcta aaggngaat tccancacac ttgcgngcgt
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576

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116

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220

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96

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334

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50

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344

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304

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250

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375

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280

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365

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425

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131

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213

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303

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356

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425

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331

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192

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438

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420cccagtttcc taataattcc acatcccaaa tgagattatc cntgccttc cagcctggaa
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179

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589

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148

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275

<210> 1048<211> 338<212> DNA<213> Homo sapien
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434

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<210> 1052<211> 243<212> DNA<213> Homo sapien
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120gtatcctctg taaaaatctg gactaaacta ttcagtcatt catggttatt cagtattcag
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243

<210> 1053<211> 156<212> DNA<213> Homo sapien
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156

<210> 1054<211> 398<212> DNA<213> Homo sapien
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120aacnccctatg agctgggact cttctgaatc aaanntaaaa aacacatatt aancactgct
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398

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397

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130

<210> 1061<211> 540<212> DNA<213> Homo sapien

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 234

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 518

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 517

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<210> 1067<211> 515<212> DNA<213> Homo sapien

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515

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353

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512

<210> 1070<211> 108<212> DNA<213> Homo sapien

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108

<210> 1071<211> 507<212> DNA<213> Homo sapien

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<210> 1072<211> 377<212> DNA<213> Homo sapien

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<210> 1073<211> 359<212> DNA<213> Homo sapien

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359

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473

<210> 1075<211> 427<212> DNA<213> Homo sapien

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<210> 1076<211> 433<212> DNA<213> Homo sapien

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<210> 1077<211> 534<212> DNA<213> Homo sapien

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534

<210> 1078<211> 537<212> DNA<213> Homo sapien

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537

<210> 1079<211> 246<212> DNA<213> Homo sapien
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246

<210> 1080<211> 220<212> DNA<213> Homo sapien
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220

<210> 1081<211> 253<212> DNA<213> Homo sapien
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253

<210> 1082<211> 223<212> DNA<213> Homo sapien
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223

<210> 1083<211> 534<212> DNA<213> Homo sapien
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420ctgttttgca nantgggatg tgggagggat tggcancct nttctcnc accgtgcagc
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<210> 1084<211> 199<212> DNA<213> Homo sapien
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<210> 1085<211> 469<212> DNA<213> Homo sapien
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469

<210> 1086<211> 199<212> DNA<213> Homo sapien
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WO 0173027

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522

<210> 1093<211> 473<212> DNA<213> Homo sapien

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<210> 1094<211> 453<212> DNA<213> Homo sapien

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414

<210> 1096<211> 546<212> DNA<213> Homo sapien

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546

<210> 1097<211> 543<212> DNA<213> Homo sapien

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409

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313

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306

<210> 1102<211> 267<212> DNA<213> Homo sapien
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267

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381

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538

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373

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223

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326

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324

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379

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120gggggggtgcc aacangaacg gagggacttg gtggcggtt gacttctcac tgnntccgc
180ttatgtaaga ancaactct caagctgtca ctctnagcat ctncgggga caggcactag
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296

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120ggatctgggg acagggtggg acgtaccatg gggacaggag gtcacagaac acacacacac
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226

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120ccacacctga gattgtggac aacaaggacg gcacggtcac tgtagatat gccccactg
180agggtcgggt ccatgagatg cacatcaaat acatgggcag ccacatccct gagagccac
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312

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120ctggtccacg aattcagttt gtgctgttta agacctggtt atttccatg agccacacat
180gcttccaatc tttcttgaag tagtgacat gttccttccc agagctagtt tctatttggc
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360tttggagttg tctccacac actcacacac acacaagcct tggacacaac natcaaaagg
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516

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120gggctgttac tgaggtagt gggccatgtg ggggtgggtg gtatacgccg gctggacgtg
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320

<210> 1120<211> 400<212> DNA<213> Homo sapien
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120tgactctgga gctgcacagc gagggcacca cgtcctgtc cttccagttc gggatgaatg
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240gagaccctgc ctttaaagct gccaacggct cctgcgagc gctgcaggcc acagtcggca
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400

<210> 1121<211> 337<212> DNA<213> Homo sapien
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60cttgataaa agttctcttg accaaagtaa cattttatcc aaaataatca tatcttagac
120cgtgtagct tgacatactt caaaaatac tgaaagtgt actgcacac agcaggctctg
180ttaataccga ggtactacat acttctcaa gacataaata gtggaagctt taagtttgat
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337

<210> 1122<211> 345<212> DNA<213> Homo sapien
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120tgacaacggg cagattgtct ctggatctcg agataaaacc atcaagctat ggaataccct
180gggtgtgtgc aaatacactg tccaggatga gagccactca gactgggtgt cttgtgtccg
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345

<210> 1123<211> 433<212> DNA<213> Homo sapien
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120aatgataaa catactggct gttgtgtgta caatgacca attgatgtgt gtgaaattgg
180aagcaaggta tgtgcaagag gtgaaataat tggcgtgaaa gttctaggca tattggtat
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433

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420gtattttcttg gnggatcaat ggggataccc gtagcnanca cacacaantt tctctttntc
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572

<210> 1125<211> 224<212> DNA<213> Homo sapien
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120aagccaactg acaaagatgc atcacgtgtc ttaggtgat gccactacc gatttgttta
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224

<210> 1126<211> 549<212> DNA<213> Homo sapien
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120tccgcctgct actgagtaag gggcattcct gttacagacc aaggagaact ggagaaagaa
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360tccgccagta tgttgtaaga aagcccttaa ataaagaagg taagaaacct aggaccaag
420cacccaagat tcagcgtctt tgttactcca cgtgtcctgc acacaaacgg gggcgtattg
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549

<210> 1127<211> 117<212> DNA<213> Homo sapien
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117

<210> 1128<211> 207<212> DNA<213> Homo sapien
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207

<210> 1129<211> 234<212> DNA<213> Homo sapien
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120ggccttgccg tgcaccttc ccacgctggt actttgacgt ggagaggaac tcctgcaata
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234

<210> 1130<211> 347<212> DNA<213> Homo sapien
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180ctcaggctgt aaaagtagct gagccatcct gccattcct ggaggctcta caggtgaaac
240tgcaggagct cagcatagac ccagctctct gggggatggt cacctggtga tttcaatgat

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347

<210> 1131<211> 546<212> DNA<213> Homo sapien

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546

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120tgtctttctt ttgggttgag ctitttttaga aagtcgaagt gaatgttg
169

<210> 1133<211> 327<212> DNA<213> Homo sapien

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240ctgtggacaa tgccgcacg gttctgcaga ttgacaatgc ccgtcttgct gctgataact
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327

<210> 1134<211> 378<212> DNA<213> Homo sapien

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378

<210> 1135<211> 547<212> DNA<213> Homo sapien

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60ttaataataa aaggtcttcc aatggaaaag tatacctacc tggggcatag aaggttgga
120gggaggagtc aagttacgga gctttgaaat tttttcatg gctttgtatg ttgaaatttg
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547

<210> 1136<211> 503<212> DNA<213> Homo sapien

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180aaaaaaccc aaaaataaaa aacaaaaaaa acaaaaaaac caagaaaaaa aaaaanagtc
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420tcaaagggcn aattccancc actggcgcc gtactannng atccnactcg gcccaacttg
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503

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96

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527

<210> 1139<211> 117<212> DNA<213> Homo sapien
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117

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255

<210> 1141<211> 224<212> DNA<213> Homo sapien
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120tcctgtctaca cctcttctt ctctccatc ctttattcag agtcatctcg cccttcccc
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224

<210> 1142<211> 337<212> DNA<213> Homo sapien
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337

<210> 1143<211> 406<212> DNA<213> Homo sapien
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240actttgtac aacaaattaa atatagtatt agattgttac ttacgtagat ttattttta
300ctatgcctta ccaagtacat ccttaacaa agtagtatgt acatgaaatt gcacttaacc
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406

<210> 1144<211> 552<212> DNA<213> Homo sapien
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552

<210> 1145<211> 344<212> DNA<213> Homo sapien
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344

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403

<210> 1147<211> 213<212> DNA<213> Homo sapien
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213

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303

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553

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311

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240ctaaaagagc acaccgtct atgtagcaaa atagtgggaa gatttatagg tagaggcgac
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133

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290

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335

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306

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372

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190

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363

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368

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103

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213

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240

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246

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268

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267

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291

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326

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331

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372

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102

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467

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176

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301

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108

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142

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108

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120gggatgcaa acgtgcaaaa gcagggggaa gctgccagg ctgagactgg agcagctagg
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<210> 1254<211> 556<212> DNA<213> Homo sapien

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556

<210> 1255<211> 494<212> DNA<213> Homo sapien

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<210> 1256<211> 312<212> DNA<213> Homo sapien

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312

<210> 1257<211> 441<212> DNA<213> Homo sapien

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441

<210> 1258<211> 287<212> DNA<213> Homo sapien

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180tccggagatg acaannagc atctggtgaa gaacacatat gttctgtgac cgccctgtct
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287

<210> 1259<211> 339<212> DNA<213> Homo sapien

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339

<210> 1260<211> 376<212> DNA<213> Homo sapien

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376

<210> 1261<211> 65<212> DNA<213> Homo sapien

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65

<210> 1262<211> 177<212> DNA<213> Homo sapien

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177

<210> 1263<211> 560<212> DNA<213> Homo sapien

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560

<210> 1264<211> 556<212> DNA<213> Homo sapien

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<210> 1265<211> 549<212> DNA<213> Homo sapien

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246

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143

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447

<210> 1269<211> 223<212> DNA<213> Homo sapien
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223

<210> 1270<211> 555<212> DNA<213> Homo sapien
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330

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262

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289

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90

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509

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381

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327

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183

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261

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184

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384

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112

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287

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382

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546

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227

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378

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541

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313

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543

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536

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539

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177

<210> 1346<211> 219<212> DNA<213> Homo sapien

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219

<210> 1347<211> 538<212> DNA<213> Homo sapien

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290

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243

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382

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412

<210> 1354<211> 85<212> DNA<213> Homo sapien

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85

<210> 1355<211> 427<212> DNA<213> Homo sapien

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427

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266

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<210> 1358<211> 102<212> DNA<213> Homo sapien
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102

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360cgggctccag cgacaggaag cggccaggt agttcatgga cctcggncgc gaccacgcta
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180g
181

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269

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124

<210> 1363<211> 276<212> DNA<213> Homo sapien
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120aagccgacca gaatgatagc cagtccctgg agctgggtgca caggttccag gactacatcg
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270

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211

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179

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241

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302

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277

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462

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133

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495

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110

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171

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494

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406

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509

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256

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441

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296

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406

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508

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539

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326

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234

<210> 1392<211> 403<212> DNA<213> Homo sapien
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259

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294

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635

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103

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384

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226

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294

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122

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451

<210> 1437<211> 721<212> DNA<213> Homo sapien

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721

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365

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294

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390

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156

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304

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297

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302

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310

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332

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541

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99

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98

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261

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77

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144

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52

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80

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42

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117

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308

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87

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373

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240gaacaagaag ttgaaggtaa acatagaata tttatacag gcactcacac ctgccatttc
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555

<210> 1540<211> 358<212> DNA<213> Homo sapien
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 240aatttctctc agaaaaatct gttagcattt cttaaaagtc cctcagattt gagggaaatt
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358

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410

<210> 1542<211> 335<212> DNA<213> Homo sapien
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 120gcatgagtac cattgatattt ggaaacttcc cagtcgtttt gtaagcttcc actgccgagg
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335

<210> 1543<211> 238<212> DNA<213> Homo sapien
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238

<210> 1544<211> 303<212> DNA<213> Homo sapien
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 180acatcaggct ggaggatttg ccccccag gactggcaaa ttacctttac tcaacatgcc
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 303

300agg

<210> 1545<211> 276<212> DNA<213> Homo sapien
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276

<210> 1546<211> 344<212> DNA<213> Homo sapien
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344

<210> 1547<211> 172<212> DNA<213> Homo sapien
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<210> 1550<211> 520<212> DNA<213> Homo sapien
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